VOLUME 4 JUNE, 1931 NUMBER 6

NATIONAL
RESEARCH COUNCIL
of CANADA

CANADIAN JOURNAL OF RESEARCH

CANADA

Published under the authority
of the
Chairman of the Committee of the
Privy Council on Scientific and Industrial Research

OTTAWA • CANADA

CONTENTS

Original Papers

Reviews and Notes

* * *

Publication and Subscriptions

The Canadian Journal of Research is published monthly by the National Research Council of Canada, Ottawa, to which address all correspondence should be directed.

The subscription rate is $3.00 per year to any part of the world. Single copies are thirty-five cents each.

Canadian Journal *of* Research

Issued by THE NATIONAL RESEARCH COUNCIL *of* CANADA

VOLUME 4 JUNE, 1931 NUMBER 6

THE INTERCHANGE OF MOLECULES BETWEEN A LIQUID AND ITS VAPOR[1]

BY T. ALTY[2] AND F. H. NICOLL[3]

Abstract

The possibility of the reflection of vapor molecules at liquid surfaces has been examined and the reflection coefficients at the surface of water and of carbon tetrachloride have been measured.

It is shown that, while there is very pronounced reflection at the surface of water, there appears to be very little, or none, at the surface of carbon tetrachloride.

Introduction

When vapor molecules strike the surface of a solid they may either be reflected immediately or they may condense on the surface, remain there for a finite time and eventually be re-evaporated from it. In the same way, vapor molecules striking a liquid surface may either enter the liquid or they may fail to do so and therefore return to the vapor phase. If the latter molecules are considered to be reflected from the surface, without entering into the question as to whether the reflection is true reflection or consists of condensation followed by subsequent evaporation, then the reflection coefficient might be defined as the ratio of the number of molecules failing to enter the liquid to the number striking its surface. From experiments such as those of Knudsen (3) on the transfer of heat to a gas from a hot tube and those of Langmuir (4) on the vapor pressure of tungsten and other metals, it appears that the amount of true reflection at the surface is quite small in the cases they investigated.

Owing to the disturbing effect of the vapor itself on the results, little work on reflection at the surface of liquids has been performed. This vapor above the surface of the liquid retards its evaporation very considerably and greatly increases the difficulty of measuring the maximum rate of evaporation from the surface.

[1] *Manuscript received February 23, 1931.*
Contribution from the Physics Laboratory, University of Saskatchewan, Saskatoon.

[2] *Professor of Physics, University of Saskatchewan.*

[3] *Graduate student and holder of a bursary under the National Research Council of Canada.*

The amount of reflection, as defined above, may be determined from the rate of evaporation of the liquid into a perfect vacuum, as may be seen at once from the following considerations.

In the case of a liquid in equilibrium with its saturated vapor, the number of molecules entering the liquid per second from the vapor will be equal to the number of liquid molecules leaving the liquid and entering the vapor. This number will not be appreciably affected by the presence or absence of the vapor above the surface, so that

Number of molecules evaporating into a vacuum per second $= N$
$=$ number leaving the liquid and entering the saturated vapor per second.
$=$ number entering the liquid from the saturated vapor per second.

Thus the number of molecules entering the liquid from its saturated vapor can be determined by a measurement of the rate of evaporation into a vacuum.

But a well-known result of the kinetic theory of gases indicates that the mass of molecules striking a surface at a temperature $T_s°K$ from a vapor at a pressure of P mm. of mercury is

$$m = 3.45\, P \sqrt{\frac{M'}{T_s}} \text{ gm. per min. per sq. cm.} \qquad (1)$$

if M' is the molecular weight of the substance and if the vapor may, to a first approximation, be considered to obey the gas laws.

It follows then that the fraction f of the incident vapor molecules which enter the liquid will be

$$f = \frac{\text{mass entering liquid}}{\text{mass striking surface}}$$

and the fraction reflected at the surface will of course be $(1-f)$.

This method has previously been employed by one of us (1) to determine the amount of reflection at the surface of water at temperatures of 18, 40 and 60° C. Those experiments indicated that surface reflection is very pronounced in the case of water, only about 1% of the incident molecules being able to penetrate the surface, the remainder being reflected.

The present work was undertaken with the object of investigating the variation of the reflection coefficient, f, with the degree of symmetry of the molecules of the liquid. For this purpose the amount of reflection from the surface of the strongly polar liquid, water, has been compared with that from the non-polar liquid, carbon tetrachloride.

The results obtained indicate that the reflection at the surface of the tetrachloride is extremely small compared with that at the surface of the water.

Experimental

The experimental arrangements were similar to those used in the earlier work, and are shown in Figure 1-*a*.

The experimental cell L is a very thin-walled glass tube of external diameter 1 cm. From its base a narrow tube T, closed at its upper end, extends upward so that the surface temperature may be measured by means of a thermocouple passing up T into the surface. The cell L is connected to a large capacity

vacuum pump through a narrow glass tube K and a vessel which contains a substance to absorb the evaporating vapor. The fine bore tube K controls the rate of pumping and hence the pressure above the evaporating surface.

The whole apparatus is immersed in a large water thermostat which is fitted with a glass front, through which the pressure measurements are made by means of a reading microscope. In the earlier work it was found advantageous to use a small mercury thermostat around the cell in order to increase as much as possible the heat transfer to the latter. It is more convenient to be able to observe the surface during an experiment and a water thermostat renders this possible. Experiment indicates that a large water thermostat with efficient stirring is almost as effective as the mercury bath in transferring heat to the cell if the glass walls of the latter are sufficiently thin. The mercury bath has therefore been discarded.

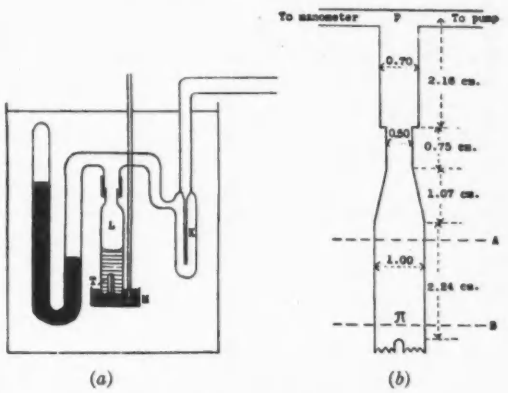


FIG. 1. *Experimental arrangements.*

It is not possible to measure directly the rate of evaporation into a vacuum. If the pressure above the evaporating surface is decreased too much, the liquid will not evaporate steadily, but boils almost explosively. For this reason a number of glass leaks, K, of different diameters, were used in turn, so that the pressure above the evaporating surface could be varied and the rate of evaporation, M, obtained as a function of this pressure.

The evaporation of the liquid through the surface will at first abstract heat from the latter more quickly than it receives heat from the body of the liquid. The surface temperature will therefore be considerably lower than that of the thermostat and the temperature difference, ΔT, between the surface and the thermostat will be greater the greater the rate of evaporation.

Since it is the *surface* temperature which will control the evaporation, it is necessary to measure ΔT. This quantity is easily obtained by allowing the liquid to evaporate in the cell L until the thermocouple sheath, T, just breaks the liquid surface. The temperature difference recorded by the thermocouple at this instant will then give ΔT directly. A copper-constantan couple was used for this purpose and ΔT was measured as a function of the pressure p above the surface.

In all these experiments it is necessary to ensure that no water from the thermostat passes up the sheath of the thermocouple, as the presence of water in varying quantity in the sheath in different experiments makes the results less consistent. Any difficulty of this nature is eliminated by the use of the

shallow mercury trough M which is just sufficiently deep to cover the base of the experimental cell and thermocouple and so is quite effective in keeping the water out of the sheath.

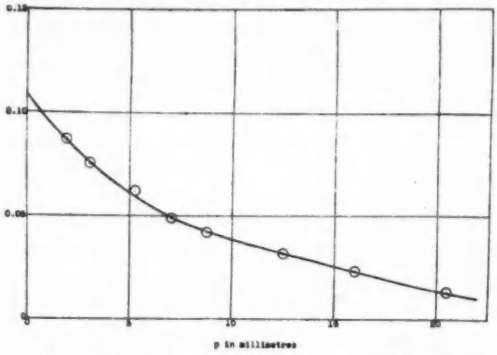


FIG. 2. *Variation of M with p for water at 30° C.*

With this apparatus the mass M evaporating per minute and the temperature difference ΔT were measured for different values of the pressure p above the liquid surface. The graphs M-p and ΔT-p so obtained for the evaporation of water in a thermostat at 30° C. are shown in Fig. 2 and 3. It will be seen that the graphs may be extrapolated to cut the axis $p=0$ at a fairly large angle so that the value of M and ΔT corresponding to $p=0$ may be obtained without great error. In the earlier work (1), on the evaporation of water, the values of M and ΔT corresponding to $p=0$ were taken as giving the rate of evaporation and temperature difference for evaporation into a complete vacuum. This involves the assumption that the pressure immediately above the surface of the liquid is equal to that registered on the manometer, an assumption which will only be true if the manometer is connected to the experimental cell very near to the evaporating surface. As the manometer connection must be an appreciable distance above this surface some error is here involved. Its magnitude will be proportional to the mass evaporating per second and in the case of water, which evaporates comparatively slowly, the error would not be expected to be large. Consequently in the earlier work the manometer was connected to the cell as near to the liquid surface as possible and the pressure difference between the vapor immediately above the surface and that given by the manometer was ignored. As a consequence the rates of evaporation at zero pressure given in that paper may be slightly too small.

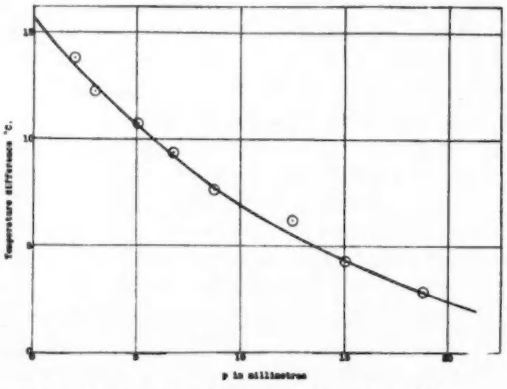


FIG. 3. *Variation of ΔT with p for water at 30° C.*

When a substance which evaporates rapidly is used, the difference between the pressure (Π) at the surface and that (p) given by the manometer cannot be ignored without further consideration. It is therefore necessary to obtain M and ΔT as functions of Π before extrapolating the curves to $\Pi = 0$ to obtain the rate of evaporation and the temperature difference for evaporation into a vacuum.

In the case of a rapidly evaporating substance another correction must also be considered. When measuring the surface temperature the level of the evaporating liquid in the experimental cell is always that of the thermocouple sheath, so that the total volume of liquid in the cell at that time is small and the temperature of the system soon attains a steady state when the pump is started.

When the mass evaporating per minute is being measured, it is necessary to keep the evaporating surface constant in area, so that the rate of evaporation per sq. cm. may later be calculated. Consequently the liquid level in L (Fig. 1-*a*) must be above the thermocouple at the end of the experiment and, therefore, very considerably above it at the beginning of the experiment. The level of the liquid in the cell may be seen from Fig. 1-*b*. In general it was at the point A at the beginning of an experiment to determine the rate of evaporation and at B when the surface temperature was being determined, so that in the former experiment there was considerably more liquid in the cell than in the latter experiment.

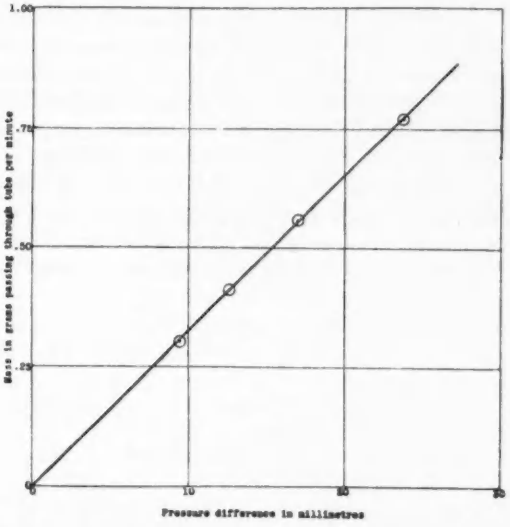


FIG. 4. *The mass of vapor passing through a given leak as a function of the pressure difference between its ends.*

The time required for the temperature of the liquid in the cell to attain a steady state will vary with the mass of liquid present. In measuring ΔT it is only necessary to begin an experiment with the liquid level a few millimetres above the thermocouple sheath, in order to be sure that the steady state is attained before the surface touches this sheath.

In measuring the rate of evaporation, however, the mass evaporated per minute before the steady state is attained will be different from, and usually greater than, its later steady value which would correspond to the value of ΔT

measured in the first experiment. It is therefore necessary to correct the rate of evaporation, M, as measured experimentally, for this variation with the mass of liquid used.

In order to investigate the nature of this correction the mass, M, of vapor flowing per minute through a given leak, K, was obtained as a function of the pressure difference p between the ends of the leak. A series of experiments were performed, the thermostat temperature being increased after each experiment. As the temperature increases the pressure registered by the manometer will of course increase and therefore the mass passing through the leak per minute will also increase. Fig. 4 shows this mass plotted against the pressure difference. It appears that for the leaks used, the mass passing through any given leak is proportional to the pressure difference between its ends and is independent of the actual mean pressure of the gas.

This relationship was found to be valid for all the leaks used, so that we may write

$$M = kp \qquad (2)$$

if k is a constant.

In the experiment to measure the rate of evaporation M, the mass evaporating per minute before the steady temperature is attained will be rather too great and the pressure as given by the manometer will be correspondingly too great. Equation (2) may however be used to correct the results for this excess evaporation and so to obtain the true rate of evaporation μ. To apply this correction, the pressure p was measured at intervals of 30 sec. from the commencement of the evaporation and the average pressure was determined. Let this be p_2 mm. and let the pressure during the experiment to determine ΔT be p_1; then the mass which would have evaporated, had the conditions of the two experiments been exactly similar throughout their course, is

$$\mu = \frac{p_1}{p_2} M$$

TABLE I

DATA OBTAINED WITH WATER AT 30° C.

Leak number	p_2 mm.	M gm. per min.	p_1 mm.	ΔT ° C.	μ gm. per min.
1*A*	20.52	0.01517	18.54	2.73	0.0137
3*A*	12.56	0.03122	12.54	6.20	0.0312
4	16.07	0.02320	15.02	4.73	0.0217
5	8.82	0.04155	8.76	7.62	0.0413
7	5.31	0.06180	5.06	10.75	0.0589
8	7.10	0.04860	6.79	9.37	0.0465
9	3.10	0.07522	2.95	12.28	0.0716
10	1.94	0.08438	2.00	13.80	0.0870

Table I gives the data obtained with water in a thermostat at 30° C. It will be observed that even in this case there was a slight difference between p_1 and p_2, p_2 usually being rather larger than p_1. This indicates that, as would be expected, the initial rate of evaporation, before the liquid had attained

a steady state, was greater than that when this steady state was established. The values of μ calculated from the measured rates of evaporation, M, are also given in Table I.

The pressure difference between the surface and the manometer may be calculated from Poisseuille's equation for the flow of a gas through a cylindrical tube. This equation, corrected for the kinetic energy of the emergent gas, may be written (2)

$$p_1^2 - p_2^2 = \frac{1}{\rho_1}\left[\frac{16\,\eta\,L\,M}{\pi r^4\left(1+\frac{4\,\xi}{r}\right)} + \frac{2M^2}{\pi^2 r^4}\right]$$

In this equation, ρ_1 is the density of the gas under a pressure of 1 dyne, L the length of tube in cm., η the coefficient of viscosity of the gas, M the mass in grams passing through per second, ξ the coefficient of slip, and p_1, p_2 the pressure in dynes at the ends of the tube of radius r cm.

Now ξ is nearly equal to the mean free path λ. Since λ/R is very small in the case of the wide experimental cell used in these experiments, the slip term may be neglected, so that if Π is the pressure immediately above the surface and p that registered by the manometer, then

$$\Pi^2 - p^2 = \frac{1}{\pi\rho_1}\left[16\,\eta\,M\frac{L}{r_4} + \frac{2M^2}{\pi}\frac{1}{r^4}\right]$$

The shape of the tube between the liquid surface and the manometer connection is shown in Fig. 1-*b*. Since this tube is not of uniform cross section the resistance terms $1/r^4$ must be summed up for the different uniform sections of the tube, so that for a tube of this type

$$\Pi^2 - p^2 = \frac{RT}{\pi M'}\left[16\eta M\,\Sigma\frac{L_1}{r_1^4} + \frac{2M^2}{\pi}\Sigma\frac{1}{r_1^4}\right]$$

since $\rho_1 = M'/RT$, if M' is the molecular weight of the gas, R the gas constant per gram molecule and T the absolute temperature of the gas.

For the case of water at 30° C. in the cell shown in Fig. 1-*b*, we have: $T = 303°\ K$, $R = 8.32\times10^7$, $M' = 18$, $\eta = 100\times10^{-6}$*, $\Sigma\frac{L_1}{r_1^4} = 453$, $\Sigma\frac{1}{r_1^4} = 414$, so that

$$\Pi^2 - p^2 = 3{\cdot}11\,\mu + 18{\cdot}8\,\mu^2 \qquad (4)$$

Π and p now being expressed in millimetres of mercury.

TABLE II

CORRESPONDING VALUES OF Π AND p

p_1 mm.	Π mm.	p_1 mm.	Π mm.
18.54	18.54	6.79	6.80
15.02	15.02	5.06	5.09
12.54	12.55	2.95	3.02
8.76	8.77	2.00	2.11

*Reference (5).

From this equation (4), together with values of μ and p_1 given in Table I, the values of Π may be calculated at once. In the case of water at 30° C. the correction $(\Pi - p)$ is extremely small, even for the largest values of μ. This will be seen from Table II which shows corresponding values of Π and p.

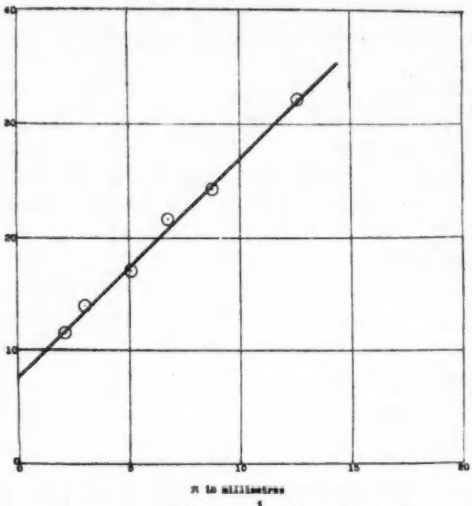


FIG. 5. *Variation of* $\frac{1}{\mu}$ *with* Π *for water.*

It therefore appears that the error involved in the earlier work on the evaporation of water (1), in which the difference between Π and p was ignored, was quite negligible.

Since Π is the pressure immediately above the surface, an extrapolation of the two curves $(\mu - \Pi)$ and $(\Delta T - \Pi)$ to cut the axis $\Pi = 0$ will give the required values of the rate of evaporation into a perfect vacuum and the surface temperature corresponding to this rate.

It appears that this extrapolation can be carried out with more accuracy if $1/\mu$ and $1/\Delta T$ be plotted against Π (Fig. 5 and 6). It will be seen that these graphs are practically linear except for pressures approaching the saturated vapor pressure. The values μ_0 and ΔT_0 corresponding to $\Pi = 0$ are therefore obtained from these curves. The values obtained in this way for the evaporation of water in a thermostat at temperature of 30° C. are: $\mu_0 = 0\,1297$ gm per min. and $\Delta T_0 = 17.9°$ C.*

Before it is possible to calculate the amount of reflection at the liquid surface it remains to estimate the area of the evaporating surface.

In order to measure this area the experimental cell, containing some of the liquid, was lowered into a rectangular glass vessel filled with the same liquid, and the meniscus was photographed through

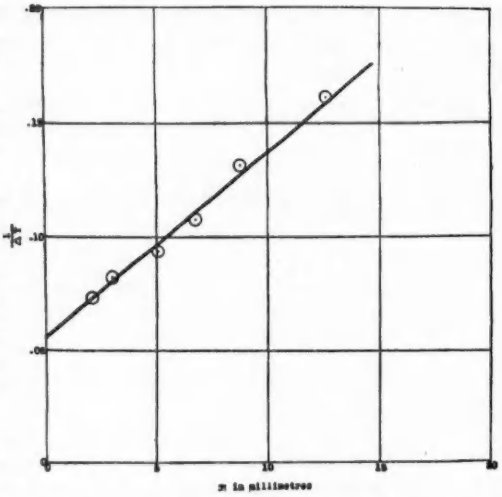
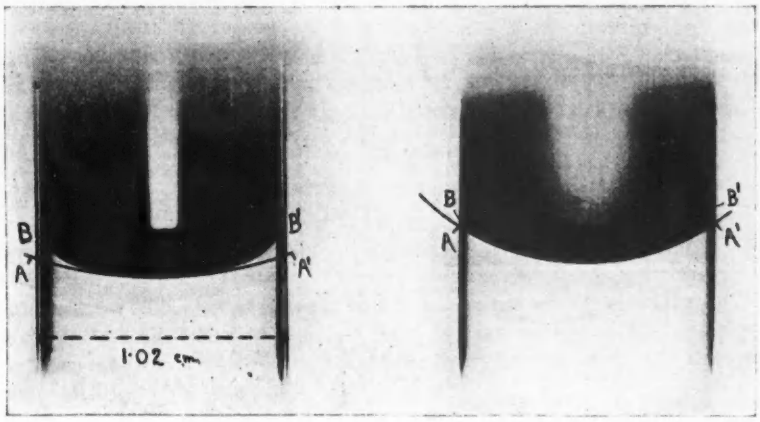


FIG. 6. *Variation of* $\frac{1}{\Delta T}$ *with* Π *for water.*

**There is a possibility that this linear relationship does not remain valid up to* $\Pi = 0$ *and that therefore the fraction* f *may be equal to unity. This is discussed in the earlier paper (1) where it is shown to be improbable.*

the liquid. Fig. 7-*a* and Fig. 7-*b* show the shapes of the meniscus in carbon tetrachloride and in water, respectively. It will be seen that the surface areas of these two liquids in the same cell are different owing to the greater concavity of the water surface.



a *b*

FIG. 7. *Photographs of the liquid surfaces.*

The area is calculated by fitting to the actual curve of the meniscus, a spherical cap having approximately the same curvature. In Fig. 7 the arc AA' is drawn so as to be of the same length as the actual meniscus BB'. It is then assumed that the actual surface area is equal to that of the cap AA'.

In the case of water in a thermostat at 30° C. we have: $A = 0.91$ sq. cm., $\mu_0 = 0.1297$ gm. per min. and $\Delta T_0 = 17.9°$ C. Therefore m_0, the mass evaporating per sec. per sq. cm. into a perfect vacuum $= \frac{\mu_0}{A} = 0.1425$ gm. per sq. cm. per min. and $T_s = (T_B - \Delta T) = 12.1°$ C. $= 285.1°$ K. Therefore $P_s = 10.50$ mm. Hence by equation (1) the mass of molecules striking unit area per minute $= m_0 = \frac{14.63 P_s}{\sqrt{T_s}} = 9.11$ gm. per sq. cm. per min. Therefore $f = \frac{0.1425}{9.11} = 1.56 \times 10^{-2}$, so that only 1.5% of the water molecules striking the liquid surface are able to enter the liquid.

This value agrees very well with the values ($f = 1.3\%$ at 40° C. and $f = 1.55\%$ at 18° C.) given in the previous paper. The essential fact, that only a small percentage of the incident molecules can penetrate the liquid surface, is unaltered by the more exact estimate of f given above.

Experiments with Carbon Tetrachloride

When carbon tetrachloride was used instead of water, it was immediately found that the rate of evaporation was greatly increased. In order to reduce the vapor pressure sufficiently the experiments were performed with a thermo-

stat temperature of +1.6° C. As in the foregoing work, the rates of evaporation (M) and surface temperature differences (ΔT) were obtained as a function of the pressure p. The results for tetrachloride are shown in Table III.

TABLE III

DATA OBTAINED WITH CARBON TETRACHLORIDE AT 1.6° C.

Leak number	p_1 mm.	M gm. per min.	p_1 mm.	ΔT °C.	μ gm. per min.
0	33.28	0.0205	32.52	0.5	0.0201
1A	23.39	0.0879	23.10	4.22	0.0689
3A	16.06	0.1850	15.68	8.13	0.1807
4	17.25	0.1625	17.13	7.28	0.1615
5	11.49	0.3029	10.80	10.55	0.2850
7	7.91	0.5991	7.43	16.38	0.5630
8	9.20	0.4393	8.84	13.02	0.4220
9	6.36	0.7784	5.87	18.70	0.7185

This table also shows the values of μ obtained from M in the manner already explained. μ is plotted against p in Fig. 8-A to make clear the difference between the experimental results obtained with water and those with tetrachloride. It will be remembered (Fig. 2) that in the case of water, the curve m-p could be extrapolated to cut the axis $p=0$ with some accuracy. In the case of the tetrachloride no pressure smaller than $p=6$ mm. could be used without causing very violent boiling of the liquid, so that the extrapolation is in this case much more uncertain.

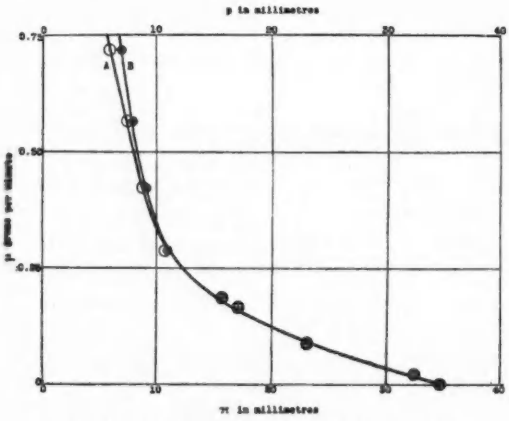


FIG. 8. *Variation of μ with p and Π for carbon tetrachloride.*

From the graphs of $(\mu - p)$ and $(\Delta T - p)$, (Fig. 8-A and Fig. 9-A), values of $(\Pi - p)$ were calculated and μ and ΔT plotted against Π as in Fig. 8-B and Fig. 9-B.

These curves are quite different from those obtained with water as the value of μ increases extremely rapidly with decrease of Π. On examining the curves $\left(\frac{1}{\mu} - \Pi\right)$ and $\left(\frac{1}{\Delta T} - \Pi\right)$, Fig. 10 and 11, it is seen that over a considerable range of pressure, these curves are linear as in the case of water. In the case of the tetrachloride, however, the linear graph intersects the Π axis instead of the axis of $1/\mu$ so that the value of $1/\mu_0$ cannot be obtained. There is some indication that the linear relationship between $1/\mu$ and Π begins to fail at the lowest pressures, as in fact it must if infinite rates of evaporation are to be

avoided. The actual curve of $\left(\frac{1}{\mu} - \Pi\right)$ would be expected to have a form similar to that of the heavy curve of Fig. 10. Its curvature near the origin cannot be very much greater than that shown, as the curve of Fig. 10 is already almost horizontal at $\Pi = 0$. This curve therefore might be taken as giving the maximum possible value of $1/\mu_0$, *i.e.*, the minimum value of μ_0. In the same way the heavy curve of Fig. 11 will give approximately the minimum possible temperature difference and hence the maximum possible surface temperature. Hence if the values of μ_0 and ΔT_0 are taken from the heavy curves of Fig. 10 and 11, the resulting value of f as obtained from equation (2) will be a minimum value. However, when f is calculated in this way, it appears that $f = 1$.

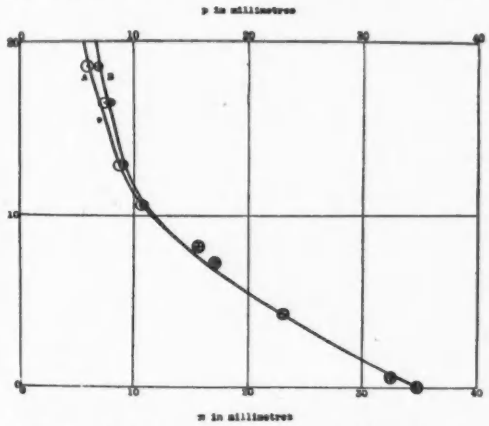


FIG. 9. *Variation of ΔT with p and Π for carbon tetrachloride.*

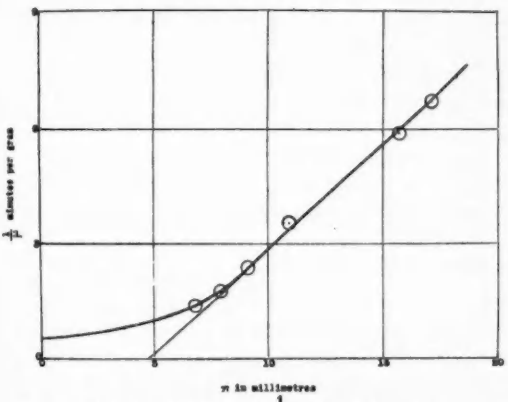


FIG. 10. *Variation of $\frac{1}{\mu}$ with Π for carbon tetrachloride.*

Hence the curves of Fig. 10 and 11 cannot intersect the $\Pi = 0$ axis at any point nearer to the origin than those shown, since such points will give values of f greater than unity, *i.e.*, more molecules enter the liquid than strike its surface.

Consequently since the true curves of Fig. 10 and 11 cannot be nearer to the origin and cannot be very much further from it than the actual curves as drawn, there can be no great error in these actual curves. They may therefore be interpreted as showing definitely that there is very little or no reflection of vapor molecules at the surface of carbon tetrachloride.

It may be added that preliminary work on the rate of evaporation of benzene, another non-polar liquid, gives results very similar to those obtained with the tetrachloride. The rate of evaporation increases with decrease of Π even more rapidly than in the case of the carbon tetrachloride. It is possible to obtain

a rate of steady evaporation of about 1 gm. per min. per sq. cm. at a surface pressure of about 10 mm. and this rate increases extremely rapidly with further decrease of pressure.

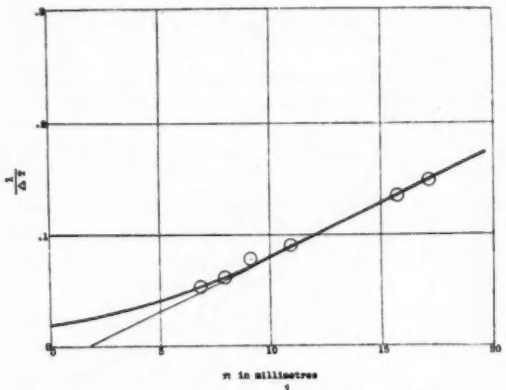


FIG. 11. *Variation of* $\frac{1}{\Delta T}$ *with* Π *for carbon tetrachloride.*

The above results indicate a very marked difference between the behavior of the polar liquid, water, and the non-polar liquid, carbon tetrachloride. In the case of the water, only a small fraction of the molecules striking the surface are able to enter the liquid, while in the case of carbon tetrachloride practically every incident molecule penetrates the liquid surface.

The work is being extended with a view to measuring the surface reflection coefficient for a number of polar and non-polar substances.

References

1. ALTY, T. (In press) Proc. Roy. Soc. Lond.
2. BENTON, A. F. Phys. Rev. 14: 403-408. 1919.
3. KNUDSEN, M. Ann. phys. 34: 593-656. 1911.
4. LANGMUIR, I. and MACKAY, G. M. J. Phys. Rev. 4: 377-386. 1914.
5. SMITH, C. J. Proc. Roy. Soc. Lond. 106A: 83-96. 1924.

THE PHOTO-ELECTRIC MEASUREMENT AND PHOTOGRAPHIC RECORDING OF DAYLIGHT[1]

By Wallace A. Thomson[2]

Abstract

A method is described by which illumination intensities were measured by a photo-electric cell and galvanometer, and a continuous photographic record obtained of the variations of intensity over a period of time during which the ground was covered with snow.

It was found that a remarkable increase in the illumination was caused by the presence of cloudiness with full sunshine. The percentage increase due to this condition in many cases was 20-30%, and on one occasion it was as high as 40%.

When there was a cloud over the sun, with most of the sky clear, the decrease in illumination was found to vary up to 35%, depending on the density of the cloud, and on many occasions it was observed that the increase in indirect illumination due to overhead cloudiness more than balanced the decrease of direct illumination when the sun was partly hidden. From this it is concluded that the intensity may be greater with the sun partly hidden than at the same time of day with a clear sky.

Introduction

The possibilities of the photo-electric cell in the measurement of daylight intensity have recently been carefully considered by several investigators, and the results that have been obtained have been very encouraging. In the opinion of the writer, the character of the information that would be of the greatest general value to those who are to apply it, would be a continuous daily record of illumination intensity. From such a record one could derive a figure representing the total amount of light received on a unit area each day in terms of foot-candle-hours, as well as the average daily or hourly intensity and the number of hours of bright sunshine.

In an endeavor to obtain such information the writer has recorded the photo-electric current furnished by a suitably exposed photo-electric cell in the manner outlined below. The investigation was conducted at the Manitoba Agricultural College, Winnipeg. Latitude 49° 50′, longitude 97° 8′.

Previous Work

An investigation of a similar nature has been conducted by Atkins and Poole (1), using a thread recorder, by Ives (4) using a recording potentiometer to determine the reduction of intensity due to smoke, by Thomson (6) using an electric clock and relay to record the hours of bright sunshine, and by Koller (5) using a recording meter for the recording of daylight.

The outstanding differences in the methods employed by various investigators are in the manner of exposing the photo-electric cell and in the method of recording the variations of the photo-electric current. In these two respects this investigation differs widely from those previously reported.

[1] *Manuscript received April 9, 1931.*
Contribution from the Physics Department, Manitoba Agricultural College, Winnipeg, Manitoba.

[2] *Lecturer in Physics, Manitoba Agricultural College.*

Apparatus

The apparatus employed was a "Visitron" type 75*A* photo-electric cell and a Leeds and Northrup d'Arsonval galvanometer with a sensitivity of 0.005 microampere. The photo-electric cell was exposed on the roof of the building and the two lead wires brought to the dark room below.

Exposure of the Cell

In a consideration of the exposure of the cell it will be seen that there is plenty of opportunity for variety. The object is necessarily to measure the illumination on a surface exposed to daylight. This surface may be horizontal, or it may face any part of the sky. The illumination of any plane surface is largely determined by the brightness of that portion of the sky which it faces. In the investigations previously mentioned (1, 4) the cell was exposed so as to measure vertical illumination. For recording the hours of bright sunshine, Thomson (6) exposed the cell so that direct light fell upon it when the sun was visible and most of the indirect light was excluded, and Koller (5) exposed the cell in an east window, but not in direct sunlight.

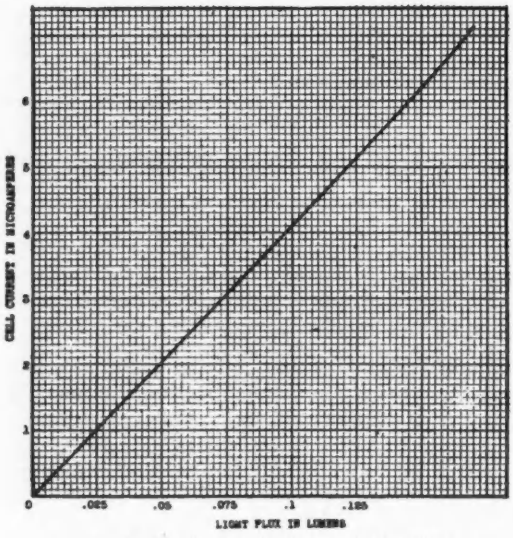


FIG. 1. *Graph showing the linear relationship between cell current and light flux at 90 volts.*

Since the indirect light forms a considerable portion of the total received on an exposed surface, it was thought by the writer that the surface on which the illumination was to be measured should be such that uniform consideration is given to both indirect and direct light. In an attempt to accomplish this the photo-electric cell was exposed in a glass hemisphere which was silvered on the inner surface so that the illumination inside would be reduced to such an intensity that the cell would have a safe operating voltage of at least 90. This silvered dome viewed from the outside has the appearance of a first class mirror, but when held to the light and viewed from the inside, objects are clearly visible through it. The uniformity of the silver coating appears to be very nearly perfect and of such a character that the shorter wave-lengths are a little more readily transmitted than the red and yellow.

By exposing the cell to natural light with and without the dome cover, and varying the voltage as required, it was possible to determine the ratio of the

transmitted to the reflected light. This ratio was found to be 1 to 320, and that when the average illumination on the exterior was 2000 foot-candles* the light flux on the cathode of the cell was 0.072 lumens.

It will be seen in Fig. 1 that the cell current in microamperes delivered by this cell is very closely proportional to the light flux, for values between zero and 0.125 lumens.

Recording Device

For about two weeks after the cell was exposed in this manner an endeavor was made to take the galvanometer readings about four times each hour, between sunrise and sunset, and at shorter intervals when the variations were rapid. These results were plotted with foot-candles as ordinates and time as abscissas and a fairly accurate record was obtained.

In the absence of a recording galvanometer or other recording device of a similar nature, the movements of the galvanometer since March 6 have been recorded photographically. A sheet of sensitized paper, 6½ by 8½ in., was mounted on a drum which was revolved by clockwork at the rate of a half-inch an hour. The image of a point source of light was focused on the paper after reflection from the galvanometer mirror. A stationary image was also produced on the paper by reflection of the same source by the galvanometer window and this image traced a base line across the lower side of the chart.

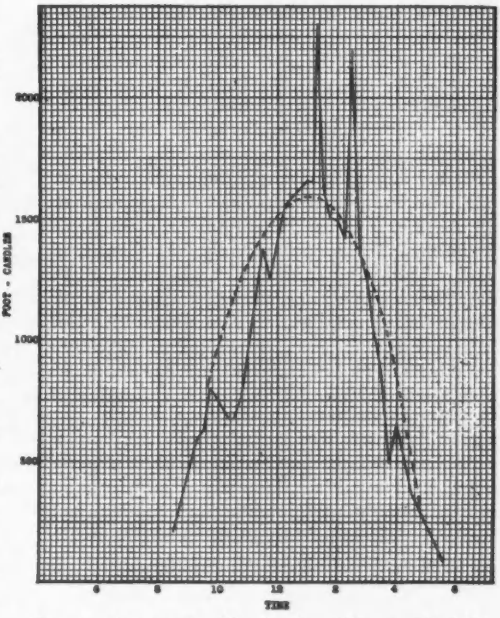


FIG. 2. *Comparative illumination, February 26, 1931, (broken line) and February 11, 1931 (continuous line).*

Results

The most important result obtained when the readings of the galvanometer were taken periodically, and which was also noticeable later in the photographic record, was the effect on the illumination produced by overhead clouds with and without the sun visible. The broken line in Fig. 2 is a record of the

**The writer has found it difficult, owing to lack of the necessary equipment, to calibrate the cell with the desired accuracy at this time. It is, therefore, desirable to consider the figures representing foot-candles illumination which correspond to certain galvanometer deflections as being approximate. Since the figures as presented in this paper are for the purpose of comparison, one day with another, any slight inaccuracy will be common to all days and will therefore not lessen their value for this purpose.*

readings taken on February 26, 1931, a day during which no clouds appeared in the sky. The continuous line in the same figure is a similar record for February 11. The sky on this day was clear from sunrise to 9:45 a.m.; patchy from 9:45 a.m. to 12:15 p.m.; clear from 12:15 p.m. to 1:15 p.m. At 1:15 p.m. the illumination was about 1650 foot-candles. A long narrow cloud came overhead from the northwest, leaving the sun visible, and the illumination increased rapidly to 2300 foot-candles, returning again to 1600 foot-candles when the cloud had passed. Another similar condition was attained at 2:15 p.m., the return to normal being at 2:45 p.m. The sun was continually visible from 12:15 p.m. to 3:00 p.m. and again from 4:00 p.m. until sunset. Between 3:00 p.m. and 4:00 p.m. a slight haze reduced the illumination slightly below normal.

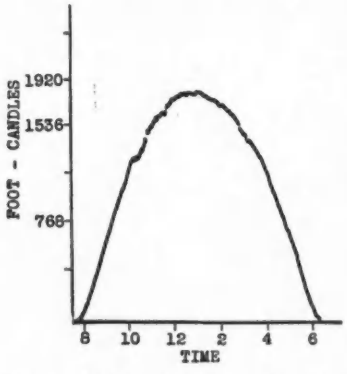


FIG. 3. *Daylight record for a clear day, March 8, 1931.*

These figures represent the rather remarkable increase of practically 40% in the illumination caused by the presence of overhead cloudiness with full sunshine. This was the extreme of many similar increases which were noted during the investigation although many as high as 20 to 30% were found to occur.

When there was a cloud over the sun with most of the sky clear, the decrease in illumination was found to vary up to 35%, depending on the density of the cloud, and on numerous occasions it was noticed that the increase in indirect illumination due to overhead cloudiness more than balanced this decrease of direct illumination when the sun was partly hidden. From this it is concluded that the illumination may be greater with the sun partly hidden than at the same time of day with a clear sky.

It should be noted that during this investigation the ground was covered with snow,. and since a considerable portion of this additional illumination would be the result of a double reflection between the snow and the cloud, the same degree of variation would not likely occur during the summer months.

Beginning on March 6 a photographic record of the variations of the photoelectric current was made each day, and it will be seen that these continuous records show the same increase above normal mentioned above.

Fig. 3 is a reproduction of the photographic record for March 8, 1931. There were no clouds in the sky at any time during the day, but a scarcely perceptible haze during the middle of the day caused the unevenness of the line and reduced the intensity very slightly. The reflecting properties of the snow had been somewhat reduced by several days of mild weather. A reproduction of this normal curve has been made with ink as a broken line in the figures following.

Fig. 4 is the chart for March 6, 1931. The sky on this day was patchy between 9:30 a.m. and 3:30 p.m., and the curve shows plainly that the intensity varied both above and below that under similar conditions with a clear sky. Between 11:30 a.m. and 3:30 p.m. the sun was at no time visible.

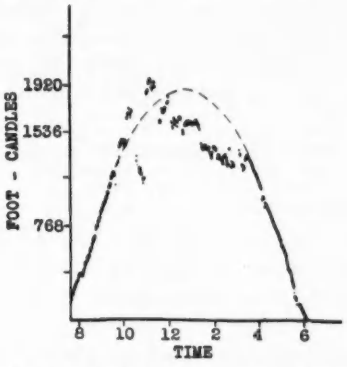


FIG. 4. *Daylight record for March 6, 1931, showing variations above and below normal caused by cloudiness.*

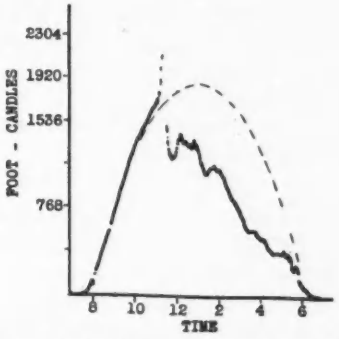


FIG. 5. *Daylight record for March 7, 1931, showing the effect of the gradual approach of a cloud bank from the north.*

Fig. 5 for March 7, 1931 shows the effect on the intensity produced by a cloud bank which gradually approached from the north. When the clouds were overhead and the sun still visible, the intensity reached a value which was 27.5% above normal, and decreased very quickly when the sun became hidden.

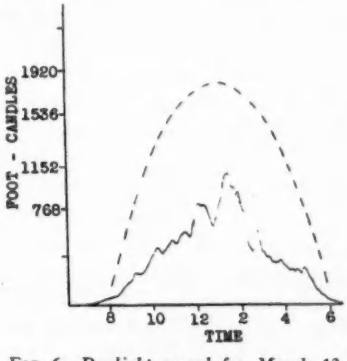


FIG. 6. *Daylight record for March 12, 1931, a day on which snow fell almost continuously.*

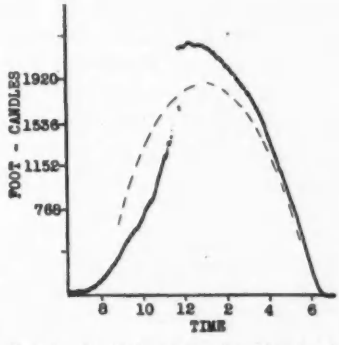


FIG. 7. *Daylight record for March 14, 1931, showing the increase of illumination by a fresh snowfall.*

Fig. 6 is the record for March 12, 1931, a day during which snow fell almost continually from dawn to dark, and shows a marked decrease of intensity.

There was very little wind accompanying the five inches of snow which fell on March 11 and 12, so that the ground was quite evenly covered with this

fresh reflecting surface. March 14 was cloudy during the morning and cleared very rapidly between 11:15 a.m. and 11:30 a.m. and remained clear until sunset. Fig. 7 is the curve for this day, showing the increase of intensity due to this fresh cover of snow.

It will be noticed that the curve in some of these records is somewhat broken. A very sudden change of intensity moved the light image across the paper so rapidly that it had insufficient time to leave a record of its path. It can, however, be readily traced by observing the position of the line at each side of the gap.

It is the intention of the writer to continue these observations throughout the summer months.

Discussion

The above results are in close agreement with the photometric observations made by Dorno (2, 3) who found that increases in the illumination up to 40% might be caused by the reflecting property of overhead clouds. From this investigation it might be expected that on a day when the sky is partially covered with small cumulus clouds, the decrease in foot-candle-hours by the loss of direct sunlight would be practically balanced by the increase of indirect light from the clouds. The majority of those who have given the matter any casual thought will be of the opinion that, with the eye as a guide, the illumination intensity on such a day with the sun visible is greater than with a clear sky. During the winter months with the ground covered with snow, an increase in this effect would be expected, and since the extent of this increase would depend largely upon the reflecting properties of the snow cover, a fresh fall of snow would affect the illumination accordingly.

It seems doubtful if any method of measuring daylight illumination which assumes that illumination and radiant heat are proportional would give the necessary consideration to the effects produced by cloudiness and a snow covered ground.

Acknowledgment

The writer desires to acknowledge his indebtedness to Dr. Alfred Savage, Department of Bacteriology and Animal Pathology, Manitoba Agricultural College, for his interest and helpful suggestions.

References

1. Atkins, W. R. G. and Poole, H. H. Nature, 125:305-306. 1930.
2. Dorno, C. Himmelshelligkeit, Himmelspolarisation und Sonnenintensitat in Davos 1911 bis 1918. Veroffentlichungen des Preusischen Meteorologischen Instituts, Nr. 303. Abhandlungen Bd. VI, Tabellen 4A und 6.
3. Dorno, C. Klimatologie im Dienste der Medizin, Vieweg, 1920.
4. Ives, J. E. U.S. Treasury Dept. Public Health Bull. No. 197. 1930.
5. Koller, L. R. Gen. Elec. Rev. 31:85-86. 1928.
6. Thomson, W. A. Can. J. Research, 4:299-303. 1931.

THE SPACE-GROUP OF POTASSIUM DITHIONATE[1]

By W. H. Barnes[2] and G. V. Helwig[3]

Abstract

An X-ray investigation of the space-group of potassium dithionate is described.

Approximate measurements of refractive indices and specific rotatory power were in harmony with those obtained by Groth. From Laue photographs and the fact of optical activity, the crystal class is trigonal trapezohedral, in agreement with crystallographic data.

The crystals showed the single cross and circular concentric rings characteristic of uniaxial crystals when plates perpendicular to the c axis were examined under crossed Nicols in convergent polarized light. The sign of the double refraction as determined with a quarter-wave plate was positive.

The primitive translations of the unit cell, determined from the a axis and c axis rotation photographs were: a_0, 9.77Å; c_0, 6.28Å, and $a:c=1:0.644$. The number of molecular units per cell was found to be three. It is deduced that the lattice must be hexagonal (Γ_h.) From the fact that no abnormal spacings of $\{0001\}$ were observed, the space-group of potassium dithionate is shown to be either D_3^1 or D_3^2.

Introduction

The dithionates, salts of dithionic acid ($H_2S_2O_6$), are a very interesting class of compounds. Crystals of some of them, such as the potassium, calcium, strontium and lead salts, are optically active (8, pp. 690-707). Others do not rotate the plane of polarized light, for example, the dihydrate and tetrahydrate of barium dithionate (8, pp. 702 and 707). Potassium dithionate crystallizes under ordinary conditions with no water of crystallization, whereas both the dihydrate and tetrahydrate of the barium salt have been prepared. The calcium, strontium and lead compounds each contain four gram-molecular weights of water for each gram-molecular weight of the corresponding salt. Crystallographically the dithionates of potassium, strontium, calcium and lead are reported to be isomorphous with each other but not with barium dithionate (8, pp. 690-707). All the dithionates, of course, contain the complex anion (S_2O_6). It is evident, therefore, that an investigation of the structures of dithionates by the methods of X-ray crystal analysis might be expected to yield interesting data from the points of view of crystal chemistry and crystal physics. With this in mind an examination of the structures of the optically active potassium, calcium, strontium and lead dithionates has been commenced.

Before investigating any detailed intensity measurements and calculations on which, of course, any progress towards the complete elucidation of the structures will depend, it has been planned to attemp first a determination of the space-group to which each salt belongs. It is hoped that similarities and differences among the collected data for a number of the dithionates will simplify any subsequent structure investigations.

[1] *Manuscript received March 19, 1931.*
Contribution from the Departments of Chemistry and Physics, McGill University, Montreal, Canada. A preliminary note on this work was read before Section III of the Royal Society of Canada in May, 1930.

[2] *Lecturer in Chemistry, McGill University.*

[3] *Graduate student, Department of Physics, McGill University and holder of a studentship under the National Research Council of Canada.*

The present paper describes the work carried out in the determination of the space group of the potassium salt, $K_2S_2O_6$.

Preparation of Dithionates

A number of methods may be employed for the preparation of individual salts of dithionic acid. The method described by Rose (9, p. 89) however is of more or less general application and has been adopted and found satisfactory for the present purpose.

This method commences with the preparation of manganese dithionate in solution by bubbling sulphur dioxide through a cold aqueous suspension of manganese dioxide. The solution is boiled to remove excess SO_2, filtered, and manganese hydroxide is precipitated by the addition of barium hydroxide. Excess barium hydroxide is removed as barium carbonate by precipitation with carbon dioxide. The solution of barium dithionate is filtered and evaporated to crystallization. The crystals of barium dithionate dihydrate ($BaS_2O_6.2H_2O$) are purified by recrystallization from distilled water and are employed for the subsequent preparation of the other dithionates.

If the cation of the dithionate, whose preparation is desired, forms a soluble sulphate (*e.g.*, potassium) warm solutions containing equivalent weights of the sulphate and of barium dithionate respectively are mixed. Barium sulphate is precipitated and the dithionate required is obtained in solution. After filtering, the solution is evaporated and the resulting crystals of the dithionate are purified by recrystallization from distilled water. Satisfactory crystals for X-ray examination are obtained by seeding a saturated aqueous solution with selected minute crystals at room temperature and allowing the solution to remain undisturbed and at an even temperature.

If the cation sulphate is insoluble, as in the case of calcium, strontium and lead, the carbonate of the appropriate cation is treated with a solution of an equivalent amount of dithionic acid. The dithionate solution is concentrated and crystals are grown in the usual way. The dithionic acid is prepared by the action of sulphuric acid on barium dithionate in solution and removal of precipitated barium sulphate by filtration.

Crystallographic Data

Groth (8, p. 690) records the following crystallographic data for potassium dithionate ($K_2S_2O_6$):

Crystal class: trigonal trapezohedral, $a:c = 1:0.6467$, density $= 2.277 - 2.280$

Optical Data

The crystals of potassium dithionate employed in the present investigation showed the single black cross and circular concentric rings characteristic of uniaxial crystals when plates perpendicular to the c axis were examined under crossed Nicols in convergent polarized light (10, p. 831). By means of a quarter-wave plate the sign of the double refraction was determined as positive (10, p. 1052).

Dr. F. F. Osborne, Assistant Professor of Geology, McGill University, kindly made some approximate measurements of the refractive indices and of the specific rotatory power and obtained values in harmony with those quoted by Groth (8, p. 690).

These data serve as a check on the identity of the crystals employed in the present X-ray investigation, confirm the uniaxial character of the crystals and verify their reported optical activity.

X-Ray Apparatus

The X-ray tube (manufactured by Hilger) was of the type developed by Shearer and described by Bragg (7, pp. 34 and 295). The potential across the tube was supplied by a 50,000-volt transformer. A tube current of about two milliamperes was employed. Laue photographs were taken with molybdenum radiation and single crystal rotation and oscillation pictures with the K_α line of copper ($\lambda = 1.54$Å).

The spectrometer was a new Bernal Universal X-ray Photogoniometer supplied by Pye (Cambridge) and described by Bernal elsewhere (2, 3, 4, 5).

X-Ray Data

Laue photographs of potassium dithionate were taken with the incident X-ray beam normal to the basal plane. Fig. 1 shows the result obtained with a target of molybdenum in the X-ray tube.

Single crystal rotation photographs about the *a* axis and *c* axis are shown in Fig. 2 and 3, respectively.

A series of 5° and 15° oscillation photographs were taken about the *a* axis for the unequivocal identification of the various orders of reflection from {0001}.

For the interpretation of the rotation and oscillation photographs the radius of the cylindrical camera employed was standardized by means of rock salt. The value obtained was 2.96 cm.

A careful study of the Laue photograph obtained with the X-ray beam perpendicular to the basal plane of a single crystal of potassium dithionate shows that the spots are distributed about three planes of symmetry perpendicular to the photographic plate intersecting in a three-fold axis of symmetry. This is the type of symmetry to be expected from a crystal belonging to any one of the classes C_{3v}, D_3 or D_{3d} since these three classes appear as D_{3d} in a Laue photograph (1, p. 247). In Wyckoff's system of symbols these classes are referred to as *3e*, *3D* and *3Di*, respectively (11, pp. 37 and 41).

The primitive translations of the unit cell were determined from the *a* axis and *c* axis rotation photographs as: $a_0 = 9.77$Å, $c_0 = 6.28$ Å, $a : c = 1 : 0.644$.

The accuracy of the measurements is of the order of 1% and the axial ratio agrees to better than 0.5% with that quoted by Groth (8, p. 690) from crystallographic data.

The volume of an hexagonal cell having these dimensions is equal to 5.19×10^{-22} cc.

Taking the density as 2.28, the molecular weight in grams as 238.0 and Avogadro's number as 6.05×10^{23}, the number of molecular units associated with each unit cell is equal to three. The actual figure obtained is 3.007, indicating satisfactory accuracy in the determination of the dimensions of the unit cell.

Space-Group of $K_2S_2O_6$

In the hexagonal and trigonal classes optical activity is shown only by substances belonging to one of the crystal classes, C_3 (trigonal pyramidal), C_6 (hexagonal pyramidal), D_3 (trigonal trapezohedral), or D_6 (hexagonal trapezohedral) (10, p. 1272). Consequently from the Laue photograph and the optical data, potassium dithionate must be classed as trigonal trapezohedral (D_3). This is in perfect agreement with the results of purely crystallographic measurements.

Examination of the seven space-groups possible in class D_3 (1, p. 249) shows that six are based on the Bravais lattice Γ_h (hexagonal) and one on Γ_{rh} (rhombohedral). It has been shown that the axial ratio agrees with that derived from crystallographic data. Assuming that the cell is hexagonal (Bravais lattice Γ_h) the number of molecular units per cell is equal to three. A rhombohedral cell with the same $c:a$ ratio would contain $\frac{8N}{3}$ molecular units, where N is equal to the number of molecules in the corresponding hexagonal cell (1, p. 246). Thus with the observed axial ratio the unit cell would contain 8 $K_2S_2O_6$ units if the Bravais lattice is Γ_{rh}. Since this number is not possible in D_3, the space group D_3^7 may be eliminated from further consideration.

Of the remaining six space-groups, all based on Γ_h, two (D_3^1 and D_3^2) give rise to no observations of abnormal spacings, while four (D_3^3 to D_3^6 inclusive) show a thirding of $\{0001\}$.

From the a axis oscillation photographs, interpreted by the Bernal method (6), all possible orders of reflections of the K_α line of Cu from the $\{0001\}$ plane from 1 to 8 inclusive have been identified, with the possible exception of (0007) which apparently is abnormally weak.

It is clear, therefore, that $\{0001\}$ is not thirded. This eliminates space groups D_3^3 to D_3^6 inclusive, leaving D_3^1 and D_3^2 as the only space groups possible for potassium dithionate. The only difference between these last possibilities with three molecular units per cell is that the diad axis, which must be associated with each molecular unit, is perpendicular to $\{10\bar{1}0\}$ in D_3^1 and to $\{11\bar{2}0\}$ in D_3^2. Data on the presence or absence of reflections from sets of general planes alone are unable to distinguish between these two arrangements.

The space-group of potassium dithionate has thus been determined as either D_3^1 or D_3^2.

Acknowledgment

The authors wish to express their thanks to Dr. R. P. D. Graham, Professor of Mineralogy, McGill University, for his assistance with certain of the crystallographic features of the work described in this paper.

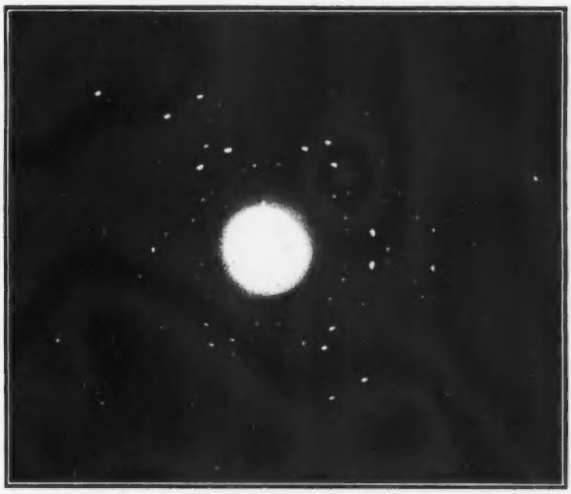


FIG. 1. *Laue photograph of* $K_2S_2O_6$ *taken with X-ray beam perpendicular to basal plane.*

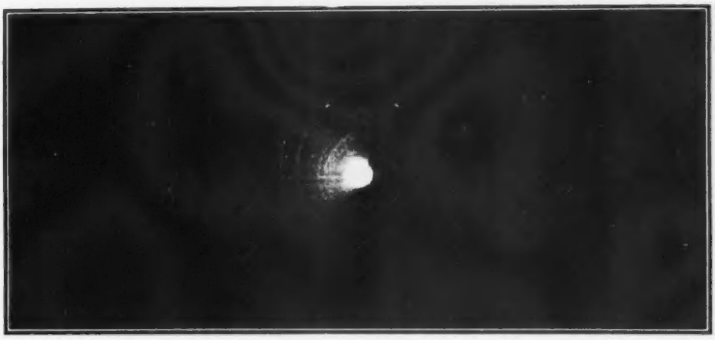


FIG. 2. $K_2S_2O_6$, *a-axis rotation photograph.*

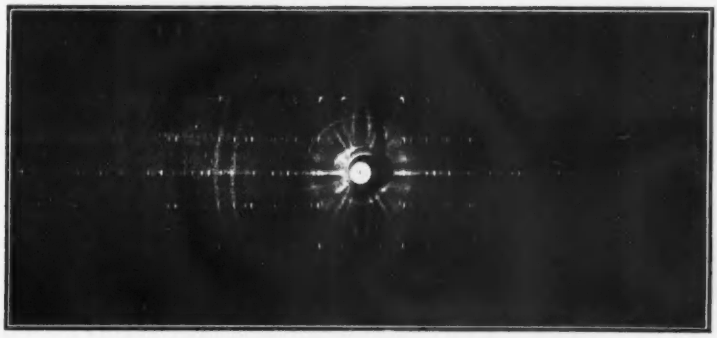


FIG. 3. $K_2S_2O_6$, *c-axis rotation photograph.*

References

1. ASTBURY, W. T. and YARDLEY, K. Trans. Roy. Soc. London, 224A: 221-257. 1924.
2. BERNAL, J. D. J. Sci. Instruments, 4: 273-284. 1927.
3. BERNAL, J. D. J. Sci. Instruments, 5: 241-250; 281-290. 1928.
4. BERNAL, J. D. J. Sci. Instruments, 6: 314-318. 1929.
5. BERNAL, J. D. J. Sci. Instruments, 6: 343-353. 1929.
6. BERNAL, J. D. Proc. Roy. Soc. London, 113A: 117-160. 1927.
7. BRAGG, W. H. and BRAGG, W. L. X-rays and crystal structure. Bell, London. 1924.
8. GROTH, P. Chemische Krystallographie, v. 2. Leipzig. 1908.
9. ROSE, H. Neus Jahrb. Min. Geol. (Beil. Bd.) 29: 53-105. 1910.
10. TUTTON, A. E.H. Crystallography and practical crystal measurement. Macmillan, v. 2. 1922.
11. WYCKOFF, R. W. G. The structure of crystals. Chem. Cat. Co. 1924.

A POSSIBLY BIOGENIC STRUCTURE IN GRENVILLE LIMESTONE IN HASTINGS COUNTY, ONTARIO[1]

By Freleigh Fitz Osborne[2]

Abstract

Certain structures in crystalline Grenville limestone resemble those ascribed to algae in late Pre-Cambrian formations. These structures are probably not biogenic and, therefore, of no value in correlation.

Introduction

Supposed fossils from the crystalline Grenville limestones of Hastings County were described many years ago: the so-called Tudor *Eozoön canadense* (2, 4, 9) was from Tudor Township, where it was found in a boulder taken from a stone fence but was later located *in situ*. The biogenic origin of *Eozoön* was an unsolved problem for many years and the subject of much controversy. The concensus of opinion (6) is that it is non-biogenic, although Schubert (8, p. 152) considers the *Eozoön*-like structures to have been made by marine plants. The new structure here described is associated with "*Eozoön*", although not the most common kind.

Locality

This structure was found at only one place in Hastings County, and Dr. F. D. Adams cannot recall having seen similar structures when mapping the area geologically. The locality is about one-half mile south of l'Amable (1) on the Hastings Road in Dungannon township. The hamlet of l'Amable is in a valley and the wall of the valley forms the steep hill up which the road ascends to the south. The structures are exposed on a south slope of a hillock, about 100 yards east of the road, which forms the highest point. The *Eozoön* is about 100 yards north of this place.

Geology

The hillock is surrounded by a covered area and crystalline limestones of typical Grenville appearance are the only rocks exposed. On the map of Adams and Barlow the area is colored as limestones with feather amphibolite. The limestone nearby has been recrystallized and has yielded to deformation by flowage. A specimen in the petrological collections of McGill University, collected by Dr. Adams from l'Amable shows a direction of shearing produced by flowage at right angles to the bedding. In other places the direction of shearing is nearly parallel to the bedding. Close to the new structures the evidence for shearing is not as great as is commonly the case. The longer axes of the structures seen in plan tend to point in the direction of flowage. The *Eozoön* exposure, on the other hand, shows evidence of considerable shearing nearly parallel to the direction of bedding. The "*Eozoön*" consists

[1] *Manuscript received April 17, 1931.*
Contribution from the Department of Geology, McGill University, Montreal, Canada.

[2] *Assistant Professor of Geology, McGill University.*

of thin alternating bands of carbonate and silicious material, and probably once formed a continuous bed. During the deformation the purer and more massive limestones yielded by flowage, whereas, the carbonate material, reinforced by the bands of silicious material, broke and was dragged out more or less parallel to the bedding to form lenticular or roughly spherical bodies.

The Structures

The structures are shown in Fig. 1 in plan where they are seen to be closely packed ellipses. The hammer is 14 in. long and about equal to the longer axis of the largest ellipse. The ellipses are marked by concentric bands of carbonate which are apparent on the weathered surface and are due to variation in the granularity of the carbonate rather than to pigmenting material. Some of the ellipses show narrow and incomplete rings of silicious material parallel to the banding, a few show it at the core, and most are outlined, at least in part, by this material. Where the silicious margin is absent the adjacent ellipses do not coalesce but are separated by a zone which weathers to depressions. No vertical section was seen and the writer cannot give a petrographic description of the structure on account of the loss of specimens. However, as is common in the Grenville, the white crystalline limestones are probably dolomitic.

Origin of the Structures

The resemblance to the markings ascribed to algae in the later Pre-Cambrian rocks first attracted attention to the structures in Hastings County. If *Collenia* or *Cryptozoön*, such as described by Walcott (11), from the Beltian of the western United States were recrystallized, the results would not be unlike the elliptical structures here described. Similar structures from South Australia and the Belchers Islands have been described as algal by Mawson and Moore, respectively. Moore's specimens were apparently *Cryptozoön*.

The specimens which resemble the structures from Hastings County most closely were brought to the writer's attention by Dr. J. E. Gill. The algal structures are of the *Collenia* type and occur in rocks supposed to be of younger Pre-Cambrian age. They were found on the Kaniapiskau River, a tributary of the Koksoak, about 25 miles above Manitou Rapids, in the old district of Ungava. They show circular to elliptical outcrops on the horizontal surface and are commonly partially or completely surrounded by rims containing silicious material, which is more resistant to weathering than the carbonate. Specimens in the laboratory show that the core is carbonate and that some layers are appreciably dolomitic as indicated by a brownish tint on the weathered surface. Some specimens show silicious bands enclosed within the outer rim. If these algae were to undergo recrystallization without flowage, the resulting structures would resemble very much those from Hastings County, even to the silicious bands.

Certain facts are against the biogenic origin of the structure. If there had been extensive flowage of the limestones as a result of deformation, the pre-existing structures would be drawn out and destroyed. The *Eozoön* a few

hundred feet away has been drawn out so that what was probably once a continuous bed has been broken. The elliptical structures have not been drawn out to any considerable extent, therefore, two explanations are possible: the particular part of the limestone containing the structures was protected from deformation in some way so that no flowage occurred, or the structures have been developed in the crystalline limestones after the flowage took place and pre-existing structures were obliterated. This latter mode of origin would explain why they are not more elongated. In view of the lack of any field evidence indicating that one part of the limestone was protected from deformation, the writer is inclined to believe that the structures are later than the flowage of the limestone, and the slight elongation of the axes of the ellipses may be explained by an initial "bias" in the direction of flowage. Recrystallization under stress with some metasomatism might produce such a structure in limestone. The exact mechanism is not clear. However, Young (12) has described and figured some very interesting and instructive examples of similar structures from dolomitic limestones in South Africa. The series is only slightly disturbed and some of the more thinly bedded members have yielded to stresses by flowage; however, in some of the heavier beds of dolomitic limestone, a structure consisting of elliptically or polygonally bounded columns with a concentric structure has been produced. In vertical section, they are seen to consist of surfaces which are convex toward the surface and, therefore, like *Collenia* (11, p. 111).

The limestone in which the change has taken place is predominantly dolomitic, and graphite was expelled from certain bands during the change. The results, however, are almost indistinguishable in the photographs from algal structures of the *Collenia* type. Young does not show how the change was brought about but suggests multilateral compression and dilatation in the limestone due to recrystallization as the source of the stresses. It is possible that Young's explanation may apply to the very similar structures in the limestone from Hastings County. They are different in that the graphite is lacking and they are in a more highly metamorphosed series.

Correlation

If the structures here described are not biogenic they have, of course, no value in correlation; if they are algal they may help to shed some light on the vexed problem of the age of the Grenville series. The resemblance to the structures in the later Pre-Cambrian rocks was pointed out, but Gruner (3) has described some algal remains from pebbles in the Ogishke conglomerate indicating the probability of algal development in the Archaean. In addition, the *Atikokania* (10) of Steeprock lake is believed to be a sponge form and therefore higher in the scale than the algae, so that the older and commonly accepted age for the Grenville series is not ruled out if the structures are biogenic.

Acknowledgment

The author is indebted to Dr. J. E. Gill for the photograph reproduced in Fig. 2.

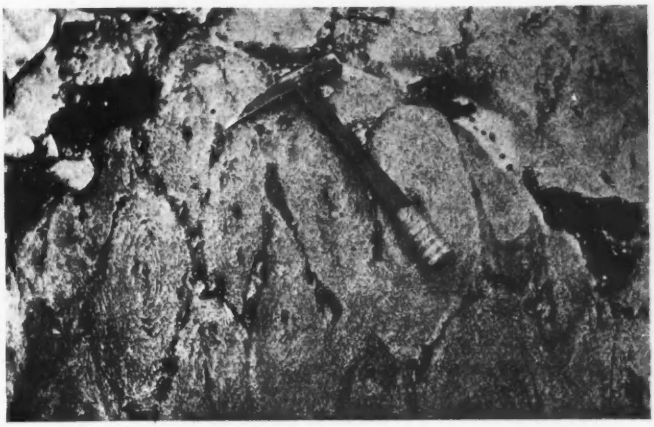


FIG. 1. *Elliptical structures in Grenville limestone near l'Amable, Ontario. The hammer is 14 in. long.*

FIG. 2. *Algae from rocks supposed to be later Pre-Cambrian age in Ungava.*

References

1. ADAMS, F. D. and BARLOW, A. E. Geol. Survey of Canada. Memoir 6. Map 770. 1910.
2. DAWSON, J. W. and CARPENTER, W. B. Am. J. Sci. 46: 245-255. 1868.
3. GRUNER, J. W. J. Geol. 31: 146-148. 1923.
4. LOGAN, W. E. Quart. J. Geol. Soc. Lond. 23: 253-256. 1867.
5. MAWSON, D. Trans. and Proc. Roy Soc. South Australia, 49: 186-190. 1925.
6. MILLER, W. G. and KNIGHT, C. W. Annual Report of the Bureau of Mines, Ontario. v. 27 part 2. 1914.
7. MOORE, E. S. J. Geol. 26: 412-438. 1918.
8. SCHUBERT, C. Historical geology. Wiley. 1924.
9. VENNOR, H. G. Geol. Survey of Canada. Report of Progress, 1866-1869. 143-171. 1869.
10. WALCOTT, C. D. Geol. Survey of Canada. Memoir 28. 16-19. 1912.
11. WALCOTT, C. D. Smithsonian Miscellaneous Collections. 64: 76-156. 1914.
12. YOUNG, R. B. Trans. and Proc. Geol. Soc. South Africa, 31: 157-165. 1928.

THE POLYMERIZATION OF ACETALDEHYDE[1]

By W. H. Hatcher[2] and B. Brodie[3]

Abstract

The polymerization of acetaldehyde to paraldehyde at 15° C. using phosphoric acid is a reaction which at low concentrations of catalyst is of the third order. At higher concentrations of catalyst the velocity tends to increase with time due to factors which may include changed ionic conditions. The velocity of the reaction is directly proportional to the quantity of added catalyst.

The method of studying this reaction was the observation of the change of volume dilatometrically with time, under conditions which would not favor the production of metaldehyde.

Historical

Acetaldehyde and its polymers, paraldehyde and metaldehyde, have been the subject of numerous investigations, most of which have been devoted to the qualitative effects of possible catalysts. The chief contribution from the physical point of view is that of Hollmann (1) who investigated the equilibrium conditions of the two-component system acetaldehyde-paraldehyde. Few data exist as to the rate at which this equilibrium is established, although practical operations have frequently demonstrated the ease with which acetaldehyde polymerizes, apparently without a catalyst, to paraldehyde, the reverse action being of negligible importance and requiring the presence of a catalyst. In this sense the word "tautomeric" used by Hollmann is misleading.

The present investigation was undertaken with a view to clarifying the mechanism by which this polymerization occurs.

Experimental

A high grade of paraldehyde was used for the preparation of acetaldehyde by distillation with a few drops of concentrated sulphuric acid; the issuing vapor was condensed in an ice-cooled spiral and collected in an ice-cooled receiver connected with a calcium chloride tube The distillate was redistilled in a similar apparatus, taking all the usual precautions necessary to obtain a pure product. All condensing and receiving vessels were subjected to cleaning by chromic acid and subsequent steaming for several hours before use.

Preliminary tests indicated the superiority of phosphoric acid as a catalyst for this study, and the absolute necessity for using freshly distilled dry acetaldehyde immediately after its preparation.

The polymerization of acetaldehyde was followed in a glass dilatometer (Fig. 1), consisting of a cylindrical bulb, closed at the lower end by a tightly-fitting stopcock, and connected with a tube terminating in a ground-glass stopper with side arm. Fastened securely to the small tube was a scale from

[1] *Manuscript received March 18, 1931.*
Contribution from the Department of Chemistry, McGill University, Montreal, Canada. Submitted by B. Brodie in partial fulfilment of the requirements for the degree of B.Sc., Honors in Chemistry.

[2] *Associate Professor of Chemistry, McGill University.*
[3] *Fourth year undergraduate, McGill University.*

a Beckmann thermometer, graduated in hundredths. The total capacity of the bulb was 25.63 cc., each small division on the scale representing 0.008 cc. Every precaution in calibration and cleanliness was strictly observed.

The catalyst was added from a 1-cc. pipette, graduated in hundredths, with a fine tip which made possible the addition of 0.01 cc. with accuracy. During an experiment the dilatometer was immersed almost to the side-arm in a carefully regulated thermostat, filled to the desired mark with acetaldehyde, and the catalyst added. Immediately the stopper was inserted, and the dilatometer inverted until thorough mixing had taken place; three such inversions were found to be sufficient, the time required being not more than one minute. The slight initial change in temperature was taken care of by the thermostat. Readings of the level of liquid in the dilatometer were made at intervals and experiments repeated several times. It required some dozen such experiments to obtain the necessary technique, an accuracy of one-third of 1% being possible.

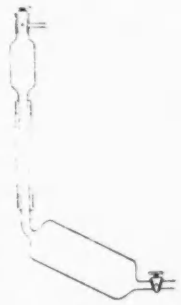


FIG. 1. *Dilatometer.*

This method, used by Turbaba (5) for finding the equilibrium concentrations of acetaldehyde and paraldehyde, depends on the change in volume when acetaldehyde passes over into paraldehyde, a loss in volume of 21.21% for 100% conversion taking place. Pascal and Dupuy (3) determined the densities of mixtures of acetaldehyde and paraldehyde at 20° C. If their values for acetaldehyde percentage be plotted against the volumes of such mixtures, a relationship will be obtained which is almost linear in nature, its greatest variation from the ideal being 0.83%.

The experiments, the results of which are given in Table I, required the densities of acetaldehyde and paraldehyde at 15° C.; using the values of Kekulé and Zincke (2) and Smits and de Leeuw (4), these are found to be 0.7870 and 0.9980 respectively at 15° C. Supposing that complete conversion to paraldehyde occurs involving a change of density from 0.7870 to 0.9980, the volume of 25.33 cc. at 15° C. used in these experiments should drop to 19.97 cc.—a loss of 5.36 cc. in volume.

On carefully adding phosphoric acid (84.3%) to acetaldehyde no shrinkage in volume for the amounts used in these experiments was discernible. The initial volume reading of aldehyde and acid can be taken therefore as the sum of the two volumes concerned. There is a limit to the quantity of phosphoric acid which may be used for polymerization purposes, as in Experiments 6, 7 and 8 it was observed that these volumes of phosphoric acid tended to become gelatinous on addition to the aldehyde, although dissolving on vigorous shaking.

A summary of the results of eight typical experiments, out of some 40 performed, is presented in Table I. Experiments 5 and 6 were continued until no further decrease in volume was observable. The final values shown represent contractions of 5.06 cc. and 5.11 cc. If 100% conversion to paraldehyde

TABLE I

SUMMARY OF RESULTS OF EIGHT TYPICAL EXPERIMENTS

Expt. No.	1	2	3	4	5	6	7	8
Volume of H_3PO_4, cc.	0.01	0.10	0.15	0.20	0.25	0.40	0.50	1.00
Time, min.	Volumes of mixed acetaldehyde and 84.3% phosphoric acid, cc.							
0	25.34	25.43	25.48	25.53	25.58	25.73	25.83	26.33
5	—	25.13	—	—	—	—	—	24.45
10	—	24.89	24.81	24.62	24.51	24.21	24.12	—
11	—	—	—	—	—	—	—	23.33
15	—	—	—	—	—	23.77	23.60	—
20	25.13	24.49	24.31	24.01	23.80	—	—	—
25	—	—	—	—	—	23.12	22.91	—
30	—	—	23.93	23.56	—	—	—	—
32	—	—	—	—	23.24	—	—	—
35	—	—	—	—	—	22.67	22.47	—
40	—	23.91	—	—	22.96	—	—	—
45	—	—	23.50	—	—	22.35	—	—
50	—	—	—	—	22.68	—	—	—
55	—	—	—	—	—	—	21.91	—
56	—	—	—	—	—	22.10	—	—
60	—	23.48	—	—	22.46	—	—	—
70	25.07	—	—	—	—	—	—	—
75	—	—	22.84	—	—	—	—	—
80	—	—	—	—	—	—	—	—
84	—	—	—	—	22.01	—	—	—
120	—	—	22.36	—	—	21.36	—	—
Final	—	—	—	—	20.52	20.62	—	—

NOTE:— *Acetaldehyde, 25.23 cc.; temperature, 15° C.*

involves a loss of 5.36 cc., these figures indicate the reaction to have progressed to an equilibrium of 94.4 and 95.3% paraldehyde.

The values given in Table I are shown graphically in Fig. 2, the numbers of the curves corresponding to the experiments similarly numbered. From these curves the figures in Table II have been obtained.

TABLE II

DATA OBTAINED FROM CURVES IN FIG. 2.

Experiment No.	2	3	4	5	6	7	8
Volume of H_3PO_4, cc.	0.10	0.15	0.20	0.25	0.40	0.50	1.00
Time to one-quarter value, min.	33	24	16½	13¼	8¼	7½	5¾
Time to one-half value, min.	—	77½	—	42¼	27	20½	9
Time to two-thirds value, min.	—	—	—	84	53¼	42	18½*

**This value was extrapolated from the curve in Fig. 2.*

Experiment 1 proceeded too slowly to admit of inclusion in the above table. The value marked with an asterisk has been extrapolated from the curve; indeed, this experiment proceeded with extreme rapidity and the agreement was not as good as in the other cases, probably due to a slightly higher local

temperature. Otherwise, since the large volume of acetaldehyde (25.33 cc.) was not materially changed by addition of phosphoric acid, it is seen that the times to fractional values were inversely proportional to the quantities of phosphoric acid added.

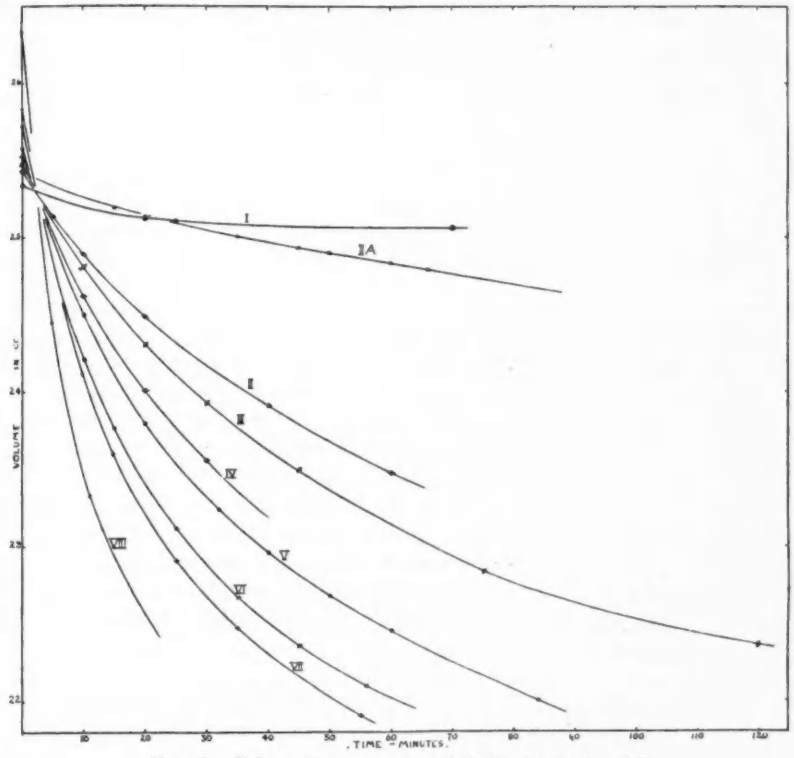


FIG. 2. *Volume-time curves (acetaldehyde-phosphoric acid).*

Following is the list of constants for intervals from the start of each experiment, omitting No. 1. These were calculated from gram-molecules of acetalde-

TABLE III

BIMOLECULAR CONSTANTS—$K \times 10^4$

Time interval, min.	Experiment No.						
	2	3	4	5	6	7	8
10	5.08	6.34	9.10	11.11	17.72	21.18	53.27
15	4.80	6.40	9.01	11.23	17.23	21.95	50.54
20	4.71	6.20	8.80	11.08	17.37	22.04	49.79
25	4.57	6.10	8.77	10.96	17.16	22.14	50.29
30	4.50	6.02	8.63	10.83	17.10	21.60	—
35	4.43	5.94	—	10.72	17.07	21.65	—

hyde per litre of reaction mixture. Since at each interval the volume had decreased to a new concentration of reagents, affecting the acetaldehyde largely and the phosphoric acid but little, a correction was made at each interval shown so as to present the concentration actually obtaining, had no change of volume occurred.

TABLE IV

TRIMOLECULAR CONSTANTS—$K \times 10^5$

Time interval, min.	Experiment No.						
	2	3	4	5	6	7	8
10	2.98	3.76	5.51	6.69	11.28	14.28	45.16
15	2.86	3.89	5.69	7.34	11.65	16.24	48.67
20	2.86	3.86	5.75	7.49	12.49	17.42	53.73
25	2.84	3.89	5.87	7.72	13.03	18.74	60.84
30	2.82	3.91	5.93	7.98	13.63	19.30	—
35	2.83	3.95	—	8.17	14.25	20.54	—

Calculations made from the first-order equation yielded no values of significance. Examination of the figures in Tables III and IV shows that at low concentrations of catalyst the reaction is definitely of the third order, but that this apparently changes with additional catalyst. Without taking into account the effect of catalyst so clearly indicated in Table II, these constants cannot give an accurate picture of the course of the reaction. Consequently these constants were multiplied by the actual volumes of the mixtures at the times represented by the constants and divided by the actual weight of pure phosphoric acid present in each experiment. This was necessitated also by the fact that in Experiment 7, say, the contraction in volume for 20 min. was far greater than in Experiment 2, as examination of Fig. 2 will show. These calculations are given in Tables V and VI.

TABLE V

BIMOLECULAR CONSTANTS × ACID FACTOR

Time interval, min.	Experiment No.						
	2	3	4	5	6	7	8
10	917	764	821	804	805	723	992
15	845	749	783	778	736	733	850
20	820	717	755	751	732	721	810
25	797	700	744	746	701	717	807*
30	775	689	725	719	698	700	—
35	758	670	—	707	691	694	—

*Extrapolated.

A comparison of these values further indicates that the reaction at low concentrations of catalyst is of the third order. In Experiment 5, however, where the volume of phosphoric acid has approached to 1% of the total volume, the trimolecular constants were increasing with time. In addition to a factor already indicated, it is probable that the ionic condition of the mixture was

TABLE VI

TRIMOLECULAR CONSTANTS × ACID FACTOR

Time interval, min.	Experiment No.						
	2	3	4	5	6	7	8
10	536	452	497	485	513	522	842
15	503	455	496	506	500	554	810
20	500	447	494	511	525	580	875
25	492	446	497	518	537	613	951*
30	480	445	497	532	555	625	—
35	484	445	—	538	567	657	—

**Extrapolated*

coming more into play. The consideration of these factors is already under way in further researches. Meantime, it was decided to test the effect of changing the concentration of acetaldehyde.

With this in view, experiments were attempted using dry ether, but with peculiar and insignificant results. With dry benzene, however, satisfactory results were obtained, and these are given in Table VII.

TABLE VII

RESULTS OBTAINED WITH DRY BENZENE

Time, min.	0	15	20	25	35	45	50	60	66	Final
Volume, cc.	25.43	25.19	25.15	25.11	25.01	24.94	24.91	24.84	24.80	22.39

NOTE:—*Benzene, 10.00 cc.; phosphoric acid, 0.10 cc.; acetaldehyde, 15.33 cc. Temperature, 15° C.*

In this experiment total conversion of acetaldehyde would have produced a volume decrease of 3.24 cc. This final value given shows the reaction to have progressed 93.6% of that possible, there being but negligible volume change on mixing the reagents. This experiment corresponds to No. 2 in Table I, and is shown in Fig. 2 by curve II*A*. Comparing the times to fractional value in these two experiments gives the following results:—

Time to one-eighth value—No. 2, 13 min.; No. 2*A*, 34 min.

Time to one-quarter value—No. 2, 33 min.; No. 2*A*, 92 min.

Since the concentration of aldehyde in No. 2*A* was three-fifths of that in No. 2, it is thus seen that the times to fractional value are inversely proportional to the squares of the concentration. This relationship is characteristic of the third-order reaction, and supports the idea that the reaction is a trimolecular one.

To judge the effect of powdered glass on this reaction, a piece of the same glass from which the dilatometer was made, was crushed, washed with chromic acid, etc., and put into the apparatus. Its volume on calibration was found to be 2.23 cc.

Experiments were then made to correspond to No. 4 and 6 in Table I at 15° C.: since the volume of acetaldehyde was necessarily less, proportionate volumes of

phosphoric acid were added, and as before readings of volume were made with time. Considerable difficulty was experienced in obtaining any readings due to intermittent bubbling from the surface of the powdered glass. The values obtained were calculated to conform with Experiments 4 and 6: in the former the velocity was about 2% slower at 10 min. and 4% slower at 30 min., while in the latter it was 7% faster at 10 min. and 5% slower at 35 min. than in the corresponding experiments without glass. The presence of glass is therefore without theoretical significance.

In preliminary experiments metaldehyde was sometimes observed after a considerable interval of time. This was not the case, however, when freshly distilled acetaldehyde was used except in the above-mentioned experiments with glass powder. Even in the latter the quantity of metaldehyde was almost negligible and the similarity in density between paraldehyde and metaldehyde and the relative insolubility of the latter would have occasioned no real error in volume.

Depolymerization of Paraldehyde

Using the same apparatus and an especially pure sample of paraldehyde the effect of phosphoric acid in producing depolymerization was examined at 15° C. A volume of 0.50 cc. of acid was not completely soluble in 22.49 cc. of paraldehyde. With what did dissolve, however, dilation ensued, the final value representing 5.6% formation of acetaldehyde.

More satisfactory results were obtained with 0.05 cc. of hydrobromic acid (48%) the final value showing 5.9%, and 5.6% acetaldehyde formed in two separate experiments. The mean of these experiments indicates 5.7% acetaldehyde formed from paraldehyde at equilibrium, while the mean of the values for the reverse process, as set forth previously, indicates the reaction to have gone 94.3% to formation of paraldehyde. As the actual dilations with different volumes of paraldehyde ranged from 0.31 to 0.34 cc., the accuracy of measurement is greatly reduced below that for the reverse action. The average of such experiments is then in good agreement with the equilibrium value obtained from experiments previously described.

Conclusion

The polymerization of acetaldehyde proceeds with a velocity which is directly proportional to the quantity of phosphoric acid employed as a catalyst. At low concentrations of catalyst this reaction is trimolecular in character; at higher concentrations, however, the order of the reaction appears to change until with the maximum of phosphoric acid (84.3%) capable of solution, *i.e.*, about 4% of the total mixture, the velocity shows decided increase with time; this may well be due to increase in the number of ions present.

The reaction comes to equilibrium representing 5.7% acetaldehyde and 94.3% of paraldehyde. It is suggested that the stability of pure paraldehyde is due to its relative immiscibility with catalysts which, present only as impurities in acetaldehyde, would readily cause polymerization of the latter.

Many interesting features have come to light which have not been mentioned in the literature, and these are being investigated at the moment.

References

1. HOLLMANN, R. Z. physik Chem. 43: 129-159. 1903.
2. KEKULÉ, A. and ZINCKE, T. Ann. 162: 125-150. 1872.
3. PASCAL, P. and DUPUY. Bull. soc. chim. 27: 353-362. 1920.
4. SMITS, A. and DELEEUW, H. L. Chem. Centr. 81: 1111. 1910.
5. TURBABA. Aus. dem Gebiet der Katalyse Tomsk. Verlag Techn. Hochschule. 1901.

THE THERMAL DECOMPOSITION OF GASEOUS PROPIONALDEHYDE ON THE SURFACE OF PLATINUM[1]

By E. W. R. Steacie[2] and Richard Morton[3]

Abstract

The thermal decomposition of gaseous propionaldehyde on the surface of platinum has been investigated in order to compare it with the homogeneous unimolecular decomposition. Unlike the decomposition of acetone and of ether, the reaction proceeds catalytically on the surface of the platinum. The course of the reaction, however, is somewhat different from that of the homogeneous decomposition. The heat of activation of the catalytic reaction is 96,500 calories, nearly double that of the homogeneous reaction. This would seem to indicate that only adsorbed molecules react, and that most of the molecules which collide with the filament are reflected from its surface without coming into thermal equilibrium with it.

Introduction

It is of considerable interest to compare the velocities of homogeneous and heterogeneous gas reactions. In this connection it has been shown by Hinshelwood (3, pp. 241-245) that there is a general tendency for homogeneous bimolecular reactions to become unimolecular on the surface of a catalyst, the heat of activation of the reaction falling to about one-half of its former value. This change in the characteristics of the reaction is accompanied by a modification of the reaction path.

The homogeneous decomposition of nitrous oxide, for example, proceeds as indicated by the equation

$$2\ N_2O \longrightarrow 2N_2+O_2.$$

The possible homogeneous unimolecular decomposition

$$N_2O \longrightarrow N_2+O,$$

followed by

$$2\ O \longrightarrow O_2,$$

is ruled out since the formation of atomic oxygen would be a highly endothermic process. The momentary concentration of a large amount of energy in the molecule would thus be necessary, and hence a very large heat of activation would be associated with the reaction. The presence of a metal surface, however, renders such a change possible, since the atomic oxygen formed can be held on the surface in an adsorbed condition and later the atoms can evaporate in pairs as molecular oxygen.

The heat of activation for the homogeneous bimolecular decomposition of nitrous oxide is 58,500 calories (4). In order that two molecules may decompose, they must therefore have a combined energy in excess of 58,500 calories. For the heterogeneous unimolecular decomposition on the surface of gold the heat of activation is 29,000 calories (5). Here one molecule in order to decompose must have an energy content in excess of 29,000 calories. The lowering

[1] *Manuscript received April 10, 1931.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada.

[2] *Assistant Professor of Chemistry, McGill University.*
Graduate Assistant in Chemistry, McGill University.

of the activation energy by the catalyst is therefore accompanied by a change in the mechanism of the reaction, in the one case only a single molecule being activated, while in the other case two molecules must be activated simultaneously. In consequence, the question of the effect of the catalyst on the magnitude of the activation energy itself is left unanswered.

It would therefore be of interest to compare the homogeneous and heterogeneous reactions in the case of a substance which decomposes homogeneously in a unimolecular manner. Previous attempts to do this have been made by Taylor (12) with acetone, and by Steacie and Campbell (11) with ethyl ether. In both cases, however, the attempt to catalyze the reaction on a hot platinum filament was unsuccessful, the reaction proceeding homogeneously in the layer of hot gas surrounding the filament.

The present communication describes a further attempt to compare a homogeneous unimolecular decomposition with the corresponding catalytic reaction, the reaction chosen for investigation being the decomposition of gaseous propionaldehyde. It may be mentioned at the outset that this reaction does proceed catalytically on the filament. The course of the reaction, however, differs somewhat from that of the homogeneous decomposition.

Apparatus

The apparatus was similar to that used by Hinshelwood and Prichard (6), and by Steacie and Campbell (11), and is illustrated in Fig. 1. It consisted of a reaction bulb *H*, about 15 cm. long, and having a capacity of about 125 cc., through which a platinum wire (0.15 mm. diameter) was sealed longitudinally. The bulb was connected by capillary tubing to a manometer *M*, and to a three-way stopcock *C*. The connecting tubing was wound with nichrome wire and heated electrically to prevent condensation of the propionaldehyde or of the products of the reaction. The tap *C* was connected to a bulb *G* containing propionaldehyde, and to the pump *I J F* by means of which the products of the reaction could be pumped into *F* and withdrawn for analysis.

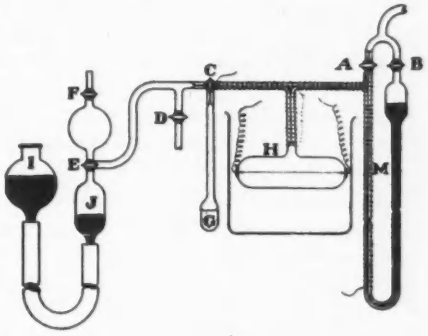


FIG. 1. *Apparatus for investigating the thermal decomposition of gaseous propionaldehyde on the surface of platinum.*

The bulb *H* was immersed in an electrically heated oil bath, the temperature of which was maintained at 45° C. In series with the filament of the bulb was a rheostat to step down the line voltage, and a precision ammeter with suitable shunts. A precision voltmeter was placed across the filament.

The whole apparatus was connected to a mercury vapor pump, backed up by a Hyvac oil pump. The apparatus could thus be evacuated to well below 0.001 mm.

Kahlbaum's propionaldehyde was used. It was redistilled and boiled between 48 and 50° C.

Experimental Procedure

The temperature of the filament was obtained from its resistance, calculated from simultaneous readings of the ammeter and voltmeter. A curve of resistance against current was plotted, and upon extrapolation to zero current the resistance at the temperature of the bath was obtained. From this value, and from the temperature coefficient of the resistance of platinum as given by Mueller (9, p. 136), and as determined by a separate experiment, the resistance of the filament at any temperature could be calculated. A curve of resistance against absolute temperature was plotted, and upon extrapolation to zero resistance was found to be in sufficient proximity to the absolute zero. At high temperatures a check on the filament temperatures was made with a Leeds and Northrup optical pyrometer. Even if the absolute temperatures of the filament are slightly in error, this will be unimportant for the present purpose, provided that the relative temperatures of various experiments are in good agreement.

Before making an experiment the whole apparatus was evacuated. Taps *A* and *B* were then closed, the desired amount of propionaldehyde vapor was admitted to the bulb, and tap *C* was closed. The initial pressure with the filament cold was then recorded. The filament was then switched on and at suitable intervals of time the pressure of the gas was noted. As the reaction proceeded, the conductivity of the resulting gases changed, usually causing a drop in voltage. The filament was maintained at the proper temperature, *i.e.*, at constant resistance, by hand regulation of the rheostat. The variation in the temperature of the filament throughout a run was not more than 3° C.

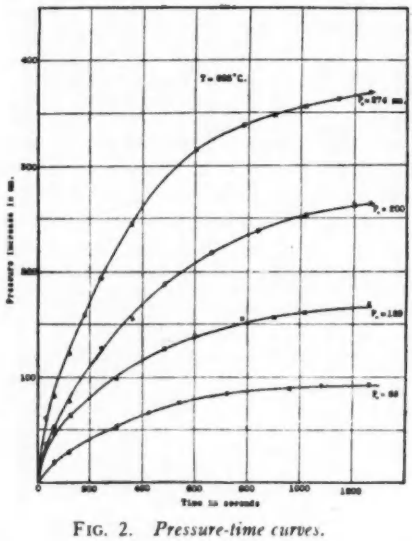


FIG. 2. *Pressure-time curves.*

Upon the completion of an experiment the pressure of the gas was observed with the filament hot, and again with the filament cold. The ratio of the two pressures allowed the pressure at the commencement of the run with the filament hot to be calculated from that observed with the filament cold.

Upon the completion of a run the gas was pumped into the bulb *F* and withdrawn for analysis.

Experimental Results

The Course of the Reaction

The simplest possible decomposition of propionaldehyde would be, $C_2H_5CHO \longrightarrow C_2H_6 + CO$. For the homogeneous decomposition Hinshelwood and Thompson (7) found the products shown in Table I. In the

present investigation, with initial pressures of 260 to 275 mm., the products shown in Table II were found.

TABLE I

DECOMPOSITION PRODUCTS OF PROPIONALDEHYDE AS FOUND BY HINSHELWOOD AND THOMPSON (7)

Temperature, °C.	CO %	CH_4 %	C_2H_6 %	$CO_2+C_2H_4$ %
506	51	18.5	26.5	2 (about)
604	44	33	18	2 (about)

NOTE:— *Initial pressure, 300 mm.*

TABLE II

THE PRODUCTS OF THE REACTION

	Temperature, ° C.		
	867	893	935
	Per cent		
CO_2	2.2	3.1, 2.3	1.3, 1.8
Unsaturated hydrocarbons	20.7	23.3, 23.3	26.6, 26.6
O_2	1.9	1.9, 1.2	1.3, 0.8
CO	37.0	37.0, 37.8	35.8, 36.2
H_2	20.7	21.0, 22.0	24.5, 22.9
CH_4	} 18.5	7.8, 6.6	5.9, 7.4
C_2H_6	}	5.8, 6.9	4.5, 3.8

NOTE:— *Initial pressure, 260 to 275 mm.*

It is thus seen that the main products of the reaction are different from those formed in the homogeneous decomposition. Carbon monoxide, however, is the principal product in both cases. It is possible, however, that the main reaction may be the same as the homogeneous decomposition, with the subsequent splitting up of most of the ethane into ethylene and hydrogen at the higher temperatures employed in this investigation (1, 2). This supposition is supported by the fact that the total pressure increase accompanying the homogeneous reaction is about 100%, while in the heterogeneous reaction the pressure increases obtained are considerably greater than this. The total pressure increases accompanying the reaction are shown in Table III.

TABLE III

PRESSURE INCREASE ACCOMPANYING THE REACTION

	At 867° C.				At 915° C.			
Initial pressure, mm.	266	197	127	66	251	197	125	77
Per cent increase	120	119	113	125	145	146	149	142
	At 893° C.				At 935° C.			
Initial pressure, mm.	274	200	129	68	265	212	152	82
Per cent increase	134	132	131	135	146	144	146	142

The Order of the Reaction

Some sample pressure-time curves are shown in Fig. 2. In Table IV the data for a typical experiment are given.

TABLE IV

DATA FOR A TYPICAL EXPERIMENT

Time, sec.	Pressure increase, mm.	Per cent decomposed	K
0	0	0	—
7	52	13.4	0.0206
14	102	26.3	0.0218
28.5	152	39.2	0.0174
51	202	52.0	0.0144
69	252	65.0	0.0152
86	302	77.8	0.0175
128	352	90.7	0.0182
300	388	100.0	—

NOTE:— *Temperature, 935° C.; initial pressure, 265 mm.*

The constant, K, shown in the last column, is that calculated for a unimolecular reaction from the expression

$$K = \frac{1}{t} \log \frac{a}{(a-x)}$$

where t is the time in seconds, a is the initial concentration (pressure) of the reactant, and x is the amount decomposed at time t.

TABLE V

TIME REQUIRED FOR 17.5 AND 35% OF PROPIONALDEHYDE TO DECOMPOSE AT VARIOUS TEMPERATURES AND INITIAL PRESSURES

	At 820° C.					At 893° C.				
Initial pressure, mm.	258	258	255	79	74	294	260	205	142	68
Time required for 17.5% of reactant to decompose, sec.	661	659	687	950	820	42	53	45	53	45
Time required for 35% of reactant to decompose, sec.	1630	1619	1770	1968	1980	122	139	122	127	140
	At 847° C.					At 915° C.				
Initial pressure, mm.	253	202	140	101	89	272	214	158	91	61
Time required for 17.5% of reactant to decompose, sec.	275	285	280	264	275	32	28	35	35	26
Time required for 35% of reactant to decompose, sec.	635	629	633	665	610	65	57	72	70	52
	At 867° C.					At 935° C.				
Initial pressure, mm.	266	202	143	108	84	272	268	206	150	79
Time required for 17.5% of reactant to decompose, sec.	104	124	106	109	107	14	10	11	10	10
Time required for 35% of reactant to decompose, sec.	297	284	294	—	244	28	25	29	19	20

A more satisfactory criterion of the reaction order is the time required for a given fraction of the reactant to disappear. In Table V the times required for 17.5 and 35% of the reactant to decompose are given for various temperatures and initial pressures. The values given are typical ones chosen from a large number of experiments.

It will be seen that the times for 17.5 and 35% reaction are independent of the initial pressure at each temperature, and hence the reaction is unimolecular. As is usual with catalytic reactions the results are slightly erratic but there is unquestionably no definite trend to the results with changing pressure. The order of the reaction is thus the same as that of the homogeneous decomposition.

The Temperature Coefficient

In Table VI the average values from all experiments of the times for 17.5 and 35% completion are given for various temperatures.

TABLE VI

TIME REQUIRED FOR 17.5 AND 35% COMPLETION OF DECOMPOSITION AT VARIOUS TEMPERATURES

Temperature, ° C.	820	847	867	893	915	935
Time for 17.5% completion, sec.	758	287	119.1	50.1	31.4	11.7
Time for 35% completion, sec.	1790	680	292	125.0	66.9	26.5

In Fig. 3, $\log t_{17.5}$ and $\log t_{35}$ are plotted against the reciprocal of the absolute temperature. The points fall well on practically parallel straight lines, indicating that the Arrhenius equation holds. From the slope of the lines in Fig. 3 the heat of activation is found to be 95,700 calories from the $t_{17.5}$ line and 97,300 calories from the t_{35} line, giving an average value of 96,500 calories.

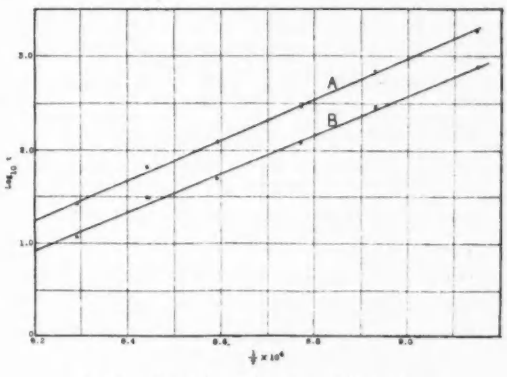


FIG. 3. *Curve A. Time for 35% reaction.*
Curve B. Time for 17.5% reaction.

Discussion

The average value obtained by Hinshelwood and Thompson (7) for the heat of activation of the homogeneous reaction was 55,000 calories. They found that the reaction proceeded unimolecularly but that the velocity constants fell off at low pressures as predicted by Lindemann's hypothesis (8). In the present investigation of the catalytic decomposition no such falling off has been noticed, the reaction proceeding unimolecularly throughout the range of pressures and temperatures used. The heat of activation for the catalytic

reaction is much higher than that of the homogeneous reaction. This differs distinctly from the results of similar investigations with acetone and ethyl ether.

Taylor (12) found that the decomposition of acetone proceeded unimolecularly and with the same heat of activation, whether carried out homogeneously or in contact with platinum. This would indicate that in the latter case the reaction proceeds homogeneously in the layer of hot gas immediately surrounding the platinum filament. A similar conclusion was drawn by Steacie and Campbell (11) from the study of the decomposition of diethyl ether in contact with platinum (and has recently been confirmed by Taylor and Schwartz (13)). In both these cases it is presumed that the energy of activation is derived by collision with the hot filament, or by radiation from it. With acetone and nickel the reaction was definitely catalytic, but the course of the reaction was entirely different from that of the homogeneous decomposition, and hence the results were not comparable.

In the case of the propionaldehyde decomposition, the products of the reaction in contact with platinum are somewhat different from those of the homogeneous decomposition. It is possible, however, that the main reaction may be the same in both cases, *viz.*, $C_2H_5CHO \longrightarrow C_2H_6+CO$. In order to obtain measurable rates, the catalytic reaction has to be investigated at temperatures about 200-300° C. higher than the homogeneous reaction. At these higher temperatures the ethane formed in the primary decomposition might itself decompose subsequently to give ethylene and hydrogen (1). This would give at least a qualitative explanation of the difference between the products of the two reactions.

The supposition might be made that the decomposition of the aldehyde is fast, while that of the ethane is slow, and hence that the real reaction under investigation is merely the slow secondary decomposition of ethane. This, however, is quite untenable since it would necessitate an immediate increase in pressure of 100% followed by a further slow increase as the ethane decomposed: whereas, in reality the pressure changes slowly and regularly at all stages of the reaction. There is therefore no doubt that it is really the decomposition of propionaldehyde which is being investigated.

The fact that the heat of activation is quite different from that of the homogeneous decomposition seems to definitely establish that the reaction proceeds heterogeneously on the surface of the platinum. In the case of a heterogeneous reaction the concept of the activation energy is somewhat complicated. The apparent heat of activation, as measured, is in error on account of the change in adsorption as the temperature changes. According to the Hinshelwood-Polany equation (3, pp. 232-234), in the case of a reaction which is not retarded by the products, the true activation energy will be greater than the apparent activation energy. This is obviously of no help in this case, since it will merely cause an increase in the discrepancy between the activation energies of the homogeneous and heterogeneous reactions. If the reaction were strongly retarded by the products, however, the true heat of activation

might be smaller than the apparent one. This cannot be the case, however, since there is no evidence of any retarding action in the experimental results. It may therefore be concluded that the difference between the heats of activation is a real one, that of the heterogeneous reaction being definitely much higher than that corresponding to the homogeneous decomposition.

This behavior is difficult to explain. The difference in the heats of activation indicates that the homogeneous reaction occurs more readily than the heterogeneous reaction, *i.e.*, a molecule needs a lower energy content in the former case. It would therefore be expected that the homogeneous reaction in the hot gas layer surrounding the filament would predominate over the surface reaction. Actually, however, the heterogeneous reaction predominates. The explanation that every molecule hitting the wire reacts cannot be valid, since this would give a low temperature coefficient depending merely on the number of molecules hitting the wire, while actually the temperature coefficient of the reaction is extremely high.

It becomes necessary to explain how a molecule can get through the hot gas layer, collide with the filament, and be reflected without reaction; while those molecules which become adsorbed are enabled to react. The most plausible assumption would seem to be that molecules which are reflected (*i.e.*, strike but are not adsorbed) never reach thermal equilibrium with the filament. If this is the case, the hot gas layer immediately surrounding the filament is at an appreciably lower temperature and conditions for the homogeneous reaction are therefore much less favorable. Those molecules which become adsorbed, however, reach thermal equilibrium and the distribution of molecular energies will be the Maxwellian distribution corresponding to the temperature of the filament.

There is a further possibility which is very unlikely, but is perhaps worth mentioning. It has been observed that the velocity constants of the homogeneous reaction fall off at low pressures, the reaction tending to become bimolecular. According to the theory of Rice and Ramsperger (10) the falling-off pressure should become higher as the temperature increases. If the real variation of the falling-off pressure were much greater than that predicted by Rice and Ramsperger, it would be possible that at the higher temperatures used in this investigation the homogeneous reaction has become bimolecular. If this were the case the heat of activation would by analogy be expected to be about doubled, *i.e.*, 110,000 calories. This is higher than that of the heterogeneous reaction, and the latter might therefore predominate.

References

1. HAGUE, E. N. and WHEELER, R. V. J. Chem. Soc. 378-393. 1929.
2. HAGUE, E. N. and WHEELER, R. V. Fuel Science Practice, 8: 560-587. 1929.
3. HINSHELWOOD, C. N. Kinetics of chemical change in gaseous systems, 2d. ed. Oxford. 1929.
4. HINSHELWOOD, C. N. and BURK, R. E. Proc. Roy. Soc. Lond. 106A: 284-291. 1924.
5. HINSHELWOOD, C. N. and PRICHARD, C. R. Proc. Roy. Soc. Lond. 108A: 211-215. 1925.
6. HINSHELWOOD, C. N. and PRICHARD, C. R. J. Chem. Soc. 127: 327-336. 1925.

7. HINSHELWOOD, C N. and THOMPSON, H. W. Proc. Roy. Soc. Lond. 113A: 221-229. 1927.
8. LINDEMANN, F. A. Trans. Faraday Soc. 17: 598-599. 1922.
9. MUELLER, E. F. International Critical Tables. McGraw Hill, v. 6. 1929.
10. RICE, O. K. and RAMSPERGER, H. C. J. Am. Chem. Soc. 49: 1617-1629. 1927.
11. STEACIE, E. W. R. and CAMPBELL, H. N. Proc. Roy. Soc. Lond. 128A: 451-458. 1930.
12. TAYLOR, H. A. J. Phys. Chem. 33: 1793-1801. 1929.
13. TAYLOR, H. A. and SCHWARTZ, M. J. Phys. Chem. 35: 1044-1053. 1931.

THE SYNTHESIS OF SOME INDOL DERIVATIVES[1]

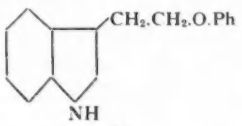
BY RICHARD H. F. MANSKE[2]

Abstract

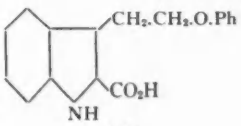
The synthesis of some indol derivatives by the well-known Fischer synthesis is described. The phenyl ether of tryptophol deserves separate mention because of its close relation to tryptophane. In all cases the now much exploited reaction of Japp and Klingemann has been utilized to prepare the requisite phenylhydrazones.

In view of the natural occurrence of a number of indol derivatives and the physiological action of some of them, it was deemed desirable to synthesize further representatives of this class, and particularly those which are related in some manner to the natural products.

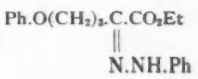
Jackson (4) has recently synthesized β-3-indolylethyl alcohol (tryptophol) which had been obtained by Ehrlich by a biological method. Its phenyl ether (I) has now been prepared by a nucleus synthetic method,



(I)

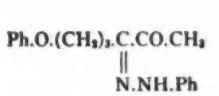


(II)

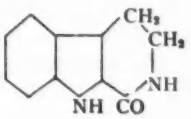


(III)

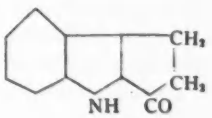
which involves the decarboxylation of the corresponding 2-carboxylic acid (II), the ethyl ester of which was obtained from ethyl α-phenylhydrazone-δ-phenoxyvalerate (III) by an application of the well-known Fischer indol synthesis. The Japp and Klingemann reaction when applied to ethyl α-acetyl-δ-phenoxyvalerate yielded the above ester phenylhydrazone by extrusion of the acetyl group when it was treated in aqueous-alcoholic alkaline solution with benzene-diazonium chloride. Conversely, if the ester was previously hydrolyzed to the acid, and the alkaline solution then treated with the diazonium salt, elimination of the carboxyl group ensued and 2-keto-3-phenylhydrazone-6-phenoxyhexane (IV) resulted.



(IV)



(V)



(VI)

The possible physiological activity of the various methoxy tryptamines made it desirable to synthesize a number of them, and the method previously described for tryptamine (5, p. 1203) and now elaborated somewhat via the

[1] *Manuscript received May 19, 1931.*
Contribution from the Department of Chemistry, Yale University, and from the National Research Laboratories, Ottawa, Canada.

[2] *Associate Research Chemist, National Research Laboratories, Ottawa.*

corresponding indol-propionic acids seemed a promising one. The decomposition of indolyl-propionazide under certain conditions gives a variable yield of di-(β-3-indolylethyl)-urea, but it has not been possible to repeat the conditions which in one case gave a yield of well over 80%. The decomposition to the urethane is much more certain but the yields are still not good.

While the work was in progress Barrett, Perkin, and Robinson (2) described the synthesis of 5- and 6-methoxy-β-(3-indolyl)-propionic acids by reactions analogous to those previously recorded (6). The properties of the entire series of compounds are in satisfactory agreement with those that had already been found. Only in the case of the dibasic acids was there appreciable disagreement, but inasmuch as the melting points of the latter are really decomposition points and depend largely on the rate of heating, the values may be expected to show considerable variation. More recently Späth and Lederer (8) synthesized 5- and 7-methoxy-tryptamine, the 6-methoxy base having been previously prepared (7, p. 124). The syntheses were therefore not carried to the final stage, and the preparation of 7-methoxy-β-(3-indolyl)-propionic acid only is detailed.

In addition, an improved procedure for the preparation of 2-keto-2:3:4:5-tetrahydro-3-carboline (V) is given and finally, the synthesis of 2-keto-dihydropentindol (VI) from the monophenylhydrazone of 1:2-diketocyclopentane is described.

Experimental Part

2-Carbethoxy-3-β-phenoxyethyl-indol

A thoroughly chilled solution of potassium hydroxide (90 gm.) dissolved in water (90 cc.) was added to an ice cold solution of ethyl α-acetyl-δ-phenoxy-valerate (105 gm) in alcohol (300 cc.). A cooled solution of benzene-diazonium chloride, prepared from aniline (40 gm.), concentrated hydrochloric acid (90 cc.) and sodium nitrite (33 gm.) was added slowly, the mixture being cooled in salt and ice. After 30 min. a slight excess of acid was added and the condensation product, (III), precipitated by the addition of much water, was extracted with ether. The dark red oil, obtained on evaporation of the dried ether solution, was heated under reflux for an hour with absolute alcohol (140 gm.) and concentrated sulphuric acid (40 gm.). The alcohol was removed at the water pump and the dark residue treated with water and a large volume of ether. A considerable portion of the ester crystallized out and was filtered off and washed alternately with water and with ether. The ethereal filtrate was washed thoroughly with water and with aqueous sodium carbonate solution, dried over sodium sulphate, and the solvent distilled. The addition of a small volume of alcohol induced almost immediate crystallization. When thoroughly cold the ester was filtered from the mother liquor and washed with cold alcohol. The total yield of the ester was 40 gm. (32% of theory). One recrystallization of the ester from a large volume of alcohol yielded colorless needles melting at 135° C.* It is sparingly soluble in alcohol, ether or benzene. Calcd. for $C_{19}H_{19}O_3N$; C, 73.79; H, 6.15; N, 4.53%. Found: C, 73.62; H, 5.78; N, 4.49%.

**Melting points are corrected.*

Hydrolysis with excess alcoholic alkali yielded on acidification 2-carboxy-3-β-phenoxy-ethyl-indol (II), which was obtained in minute needles by cautiously adding benzene to a hot concentrated alcoholic solution, m.p. 166-167° C. Calcd. for $C_{17}H_{15}O_3N$; N, 4.98%. Found: N, 5.01%.

When the acid was heated to 200° C., preferably with finely divided copper, carbon dioxide being eliminated, and the residue distilled under reduced pressure, a colorless oil was obtained which crystallized completely on cooling. It was recrystallized from benzene-petroleum ether and then consisted of flat colorless needles melting sharply at 99° C. It gives a brilliant red color with Ehrlich's reagent. Calcd. for $C_{16}H_{15}ON$; N, 5.11%. Found: N, 5.66%.

2-Keto-3-phenylhydrazone-6-phenoxyhexane (IV)

A mixture of 20 gm. of ethyl α-acetyl-δ-phenoxyvalerate and 5 gm. of potassium hydroxide in 30 cc. of 50% alcohol was allowed to remain on ice overnight. The mixture was diluted with much water and some unsaponified ester extracted with ether. There was then added a solution of benzene-diazonium chloride prepared from 7 gm. of aniline, together with a large excess of sodium acetate. The separated brown oil was thoroughly washed in ethereal solution first with dilute acid and then with dilute alkali. The ether was removed from the dried solution and the residue, distilled in vacuum, yielded a pale viscous oil which crystallized in the course of several days. Adhering oil was removed on porous tile and the substance was recrystallized by the cautious addition of petroleum ether to a benzene solution. It was obtained in stout pale yellow needles, m.p. 110° C. The yield of purified product was 3.5 gm. Calcd. for $C_{18}H_{20}O_2N_2$; C, 72.97; H, 6.76; N, 9.46%. Found: C, 73.71; H, 6.74; N, 8.65%.

Ethyl 2-Carbethoxy-7-methoxy-β-(3-indolyl)-propionate

The original procedure of Manske and Robinson (6) for the preparation of the corresponding desmethoxy-compound, which incidentally has not been improved upon materially in spite of numerous attempts, when applied in this case gave the substance in 37 to 40% yield. The quantities employed were:—ethyl cyclopentanone-carboxylate, 63 gm ; potassium hydroxide, 50 gm.; *o*-anisidine, 49 gm. diazotized in 90 cc. of concentrated hydrochloric acid with the requisite amount of sodium nitrite, and sufficient ice to keep all solutions cold. The dark brown oily phenylhydrazone obtained on acidifying the solution was washed by decantation with cold water, and dried at room temperature. In the course of several days it crystallized for the greater part. Esterification and ring closure were effected by heating under reflux for 40 min. with 250 cc. of absolute alcohol and 55 cc. of concentrated sulphuric acid. The ester isolated in the usual way was obtained as a pale yellow distillate (b.p. 225-243° C./8 mm.) which crystallized for the greater part on cooling. A small amount of oil was removed by washing with benzene-petroleum ether (1:1) and the ester was purified by recrystallization either from benzene or alcohol; m.p. 95-96° C. Calcd. for $C_{16}H_{21}O_5N$; C, 63.95; H, 6.58%. Found: C, 63.91; H, 7.20%.

The corresponding dibasic acid prepared in the usual way by hydrolysis with alcoholic alkali was recrystallized from hot 95% alcohol, in which it is sparingly soluble when cold. It melts at 232° C. with elimination of carbon dioxide. Calcd. for $C_{12}H_{13}O_5N$; N, 5.33%. Found: N, 5.51%.

7-Methoxy-β-(3-indolyl)-propionic acid

The purified dibasic acid admixed with a small amount of copper powder was heated in an oil bath at 235-240° C. until evolution of carbon dioxide ceased. Solution of the residue in aqueous sodium carbonate and reprecipitation followed by recrystallization from 20% alcohol and finally from hot water, in which it is sparingly soluble, yielded large colorless rhombic plates of the acid melting at 146° C. Calcd. for $C_{11}H_{13}O_3N$; N, 6.39%. Found: N, 6.55%.

Di-β-(3-indolylethyl)-urea

The azide of indolylpropionic acid (6) when decomposed with hot water yielded a resinous material which, when freed of basic and acidic impurities by washing with acid and with alkali successively and triturated with a small volume of alcohol, frequently gave a small amount of the urea. Recrystallization from hot alcohol yielded colorless needles, melting at 159° C. Calcd. for $C_{21}H_{22}ON_4$; C, 72.8; H, 6.4; N, 16.2%. Found: C, 73.3; H, 6.5; N, 16.0%.

N-Carbomethoxy-β-(3-indolylethyl)-amine

The azide, prepared from 10 gm. of β-(3-indolyl)-propionazide and dried first by suction on a funnel and then in a desiccator in a high vacuum, was suspended in 75cc. of anhydrous methanol and the mixture heated, gently at first and finally under reflux for several hours. The greater portion of the solvent was distilled off. On cooling a small quantity, a sparingly soluble crystalline substance frequently separated which was washed with cold alcohol and recrystallized from a large volume of boiling alcohol. It was thus obtained in flat slender needles melting at 237° C. The substance is neutral and gives with Ehrlich's reagent an orange red color. Analysis showed it to be di-indolylpropionhydrazide, $C_8H_6N\ CH_2.CH_2.CO.NH.NH.CO.CH_2.CH_2.NC_8H_6$. Calcd. for $C_{22}H_{22}O_2N_4$; C, 70.59; H, 5.88; N, 14.97; mol. wt. 374. Found: C, 70.45; H, 5.76; N, 14.93%; mol. wt. 435 (Rast).

The mother liquor from the above substance was evaporated to a brittle resin in vacuum and extracted with several successive portions of ether. The ethereal solution on evaporation to a syrup yielded on cooling a solid crystalline mass. Adhering oil was removed on porous tile and the substance recrystallized from a concentrated ethereal solution by cautious addition of petroleum ether. The urethane was obtained in colorless plates of a pearly lustre melting at 82° C. Calcd. for $C_{11}H_{14}O_2N_2$: C, 66 05; H, 6.42; N, 12.84%. Found: C, 66.15; H, 6.44; N, 12.82%.

For the conversion of the urethane via the phthalimide (5) into the amine, it was not necessary to purify the former. It sufficed to remove volatile products from the crude urethane at 100° C. and treat the residue from 10 gm. of hydrazide with an equal weight of phthalic acid or anhydride, and heat the

mixture in an oil bath to 230° C. until evolution of carbon dioxide ceased. The phthalimido compound was isolated from the reaction product in the usual way (yield, 3.3-4.8 gm.) and converted into the free base by means of hydrazine hydrate.

2-Keto-2:3:4:5 Tetrahydro-3-carboline (V)

The azide prepared from 6 gm. of the hydrazide was thoroughly washed with water and drained as far as possible on a Buchner funnel. It was then suspended in 60 cc. of 80% acetic acid and the mixture gradually heated. The elimination of nitrogen and ring closure were completed on boiling the solution for a short while. A large volume of water was added and the precipitated oil washed by decantation with water. It readily crystallized and was then washed thoroughly with aqueous sodium carbonate to remove acidic impurities. Final washing with water and with 30 cc. of 50% alcohol yielded 3.9 gm. of the product melting at 174° C. After one recrystallization from alcohol it melted at 185° C. (1).

2-Keto-dihydropentindol (VI)

A solution of 19 gm. of cyclopentan-1:2-dione (3) monophenylhydrazone in 50 cc. of alcohol was heated to boiling and treated with 50 cc. of concentrated hydrochloric acid. The mixture was then heated on a steam bath for 30 min. and diluted with a large volume of hot water. The precipitate, which assumed a granular form was washed on a Buchner funnel first with hot water, then with aqueous sodium hydroxide, more hot water and finally with alcohol in which it is sparingly soluble. It was recrystallized from a large volume of hot acetic acid and was thus obtained in short stout prisms, melting at 248-249° C. Yield, 7 to 9 gm. Calcd. for $C_{11}H_9ON$; C, 77.2; H, 5.3; N, 8.2%. Found: C, 77.5; H, 5.0; N, 8.6%.

References

1. Ashley, J. N. and Robinson, R. J. Chem. Soc. 1376. 1928.
2. Barrett, H. S. B., Perkin, W. H. and Robinson, R. J. Chem. Soc. 2942-2946. 1929.
3. Dieckmann, W. Ann. 317: 27-109. 1901.
4. Jackson, R. W. J. Biol. Chem. 88: 659-662. 1930.
5. Manske, R. H. F. J. Am. Chem. Soc. 51: 1202-1204. 1929.
6. Manske, R. H. F. and Robinson, R. J. Chem. Soc. 240-242. 1927.
7. Späth, E. and Lederer, E. Ber. 63: 120-125. 1930.
8. Späth, E. and Lederer, E. Ber. 63: 2102-2111. 1930.

STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES.

XXXVI. STRUCTURE OF THE LEVAN SYNTHESIZED BY THE ACTION OF BACILLUS SUBTILIS ON SUCROSE[1]

By Harold Hibbert[2] and Fritz Brauns[3]

Abstract

The action of *B. subtilis* on a sucrose nutrient medium gave a 2:6 anhydro fructofuranose polysaccharide, levan, identical in structure with that synthesized by the action of *B. mesentericus*. The yield of levan (calculated on the fructose portion of the sucrose taken) amounted to 60-65%. The pure, ash-free, snow-white, powdery levan on hydrolysis with oxalic acid, was converted into fructose, the yield of the pure, crystalline hexose amounting to 99%. On methylation the levan was converted into trimethyl levan, the yield of partially purified product amounting to 88.5%, that of the highly purified material being 75%. The trimethyl levan on hydrolysis was converted into 1:3:4 trimethyl fructofuranose identical with that obtained from the levan synthesized by *B. mesentericus* from the same sucrose nutrient medium. The pure, snow-white, crystalline 1:3:4 trimethyl fructofuranose was obtained in a yield of 98.5%.

Attention is drawn to the marked influence exerted on the course of the methylation by small amounts of inorganic impurities present in the levan and to the necessity for using only highly purified, ash-free products for physico-chemical investigations on the properties and structure of polysaccharides. It would appear that insufficient purification of many of the products, such as starch and inulin, used previously by different investigators, renders somewhat uncertain various conclusions drawn regarding the structure of these substances.

Introduction

In a previous communication (7) it was shown that the polysaccharide, levan, formed by the action of *B. mesentericus* on sucrose is a polymerized 2:6 anhydro fructofuranose. Due to the close generic relationship (1) known to exist between *B. mesentericus* and *B. subtilis* it seemed highly probable that the levan synthesized by the latter organism from a sucrose nutrient medium possesses the same structure, and a careful investigation of the highly purified product has shown this to be the case.

Due to improvements effected in the technique of its isolation, purification, methylation and hydrolysis, it has been possible to obtain very high yields in all of these operations. This is regarded as a necessary procedure inasmuch as it is intended to carry out various physico-chemical investigations on the nature of "supermolecular products", and a considerable amount of the work of previous investigators on such derivatives as starch and inulin would seem to be vitiated by the fact that insufficiently purified products were employed. Thus it has recently been shown (9) that inulin, purified by the usual methods, contains 5% of a mixture of difructose anhydrides and about 3% of aldose constituents, a fact which would indicate the advisability of exercising considerable caution in dealing with experimental results based on the use of such material.

[1] *Manuscript received April 24, 1931.*

Contribution from the Industrial and Cellulose Chemistry Laboratories, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada and the Canadian Pulp and Paper Association.

[2] *Professor of Industrial and Cellulose Chemistry, McGill University.*

[3] *Research Assistant, Division of Industrial and Cellulose Chemistry, McGill University.*

Relation of B. subtilis to B. mesentericus

A full description of *B. mesentericus* was given in a previous communication by Harrison, Tarr and Hibbert (4).

Both *Bacillus mesentericus* and *Bacillus subtilis* may be regarded as fairly closely related organisms inasmuch as both have been classified by the Society of American Bacteriologists under the same genus. However these bacilli differ in certain respects. Thus the rods of *B. subtilis* are swollen at sporulation, while those of *B. mesentericus* do not become affected in this manner. Also, the two species exhibit somewhat different cultural characteristics, *B. mesentericus* forming a characteristic "mesentery-like" growth which slowly becomes brown, when the organism is cultivated on potato, while *B. subtilis*, when cultivated on the same medium, gives rise to a flat, smooth, adherent growth, which spreads over the entire surface and exhibits a pinkish tinge after a few days' incubation.

The organisms employed were of the same strains studied in a previous investigation (4), and both agreed with the description given by the Society of American Bacteriologists for this genus. The *B. mesentericus* differed from the species described by the Society in the fact that it reduced nitrates to nitrites.

The Structure of Levan Formed from Sucrose by B. subtilis

The technique employed, with various modifications, was essentially that used in the previous communication (7). It was found advisable to precipitate the levan from the aqueous solution with methyl rather than with ethyl alcohol, since the sucrose and other sugars present are more soluble in the former solvent. An ash-free product was obtained by electrodialysis of the aqueous solution in the Pauli apparatus (10) followed by concentration of the solution and reprecipitation with methyl alcohol. It is very important to remove all of the inorganic impurities in this manner, since otherwise difficulties are encountered in the later methylation process. Even when the ash-content of the levan is not more than 0.5%, the methylated product (obtained by the action of the dimethyl sulphate and alkali) is insoluble in chloroform and methyl iodide and cannot be methylated further with silver oxide and methyl iodide (Purdie's reagent)*. No difference was found in the

*Similar difficulties are encountered in the methylation of cellulose. According to Freudenberg and coworkers (2, 3) the complete methylation of cellulose in two stages with caustic soda and dimethyl sulphate is possible only provided that in the first step a methoxyl value greater than about 38% can be obtained. With a lower methoxyl content (30%) the cellulose material acquires a slimy character and, in this condition, further and complete methylation is either impossible, or only takes place with very great difficulty. The reason given for this remarkable difference in behavior is the assumed formation of soluble lower methylated products. This explanation appears far from satisfactory and the inability to state the experimental conditions on which the success of the methylation depends points to the desirability of a more thorough investigation of this reaction. It is the authors' intention to investigate this problem.

Preliminary work indicates that the methylation proceeds much more readily when the cellulose is employed in a finely divided state, or when cellulose acetate is employed for the purpose of ensuring a complete methylation of the cellulose. Since forwarding the manuscript for publication a valuable research by Haworth, Hirst and Thomas (8) has appeared, in which they arrive at the same result. They show that cellulose acetate can be converted into a fully methylated cellulose by two successive treatments with dimethyl sulphate and sodium hydroxide. (H. H.)

TABLE I

COMPARISON OF THE PROPERTIES OF SYNTHETIC LEVAN OBTAINED BY THE ACTION OF B. SUBTILIS AND B. MESENTERICUS (4) RESPECTIVELY ON SUCROSE

Levan prepared by action of *B. subtilis*				
Properties of the snow-white, ash-free levan	Fructose obtained by hydrolysis of the pure levan	Pure, snow-white ash-free trimethyl levan	Pure crystalline 1:3:4 trimethyl fructofuranose	Pure levan acetate
$[\alpha]_D^{22} = -46.1°$ (water) (c = 5.88)	$[\alpha]_D^{22} = -90.5°$ (water) (c = 4.8)	$[\alpha]_D^{21} = -88.0°$ (tetrachloroethane) (c = 3.57)	$[\alpha]_D^{22} = +21.55°$ (tetrachloroethane) (c = 3.56)	$[\alpha]_D^{22} = +9.5°$ (tetrachloroethane) (c = 4.58)
Yield: (calcd. on the fructose part of the sucrose molecule), 60-65%	Yield: (snow-white crystalline fructose), 99.1%	Yield: (partially purified), 88%; (highly purified), 75%	Yield: 98.5%, m.p. 74° C. (a). Mixed m.p. of (a) and (b) 74° C.	Yield: 87%. Product sinters at 103° C.—only completely melted at 133°C.
Levan prepared by action of *B. mesentericus* (4)				
$[\alpha]_D^{22} = -45.3°$ (water) (c = 2.2)	$[\alpha]_D^{21} = -90.75°$ (water) (c = 1.18)	$[\alpha]_D^{23} = -87.3°$ (tetrachloroethane) (c = 2.75)	$[\alpha]_D^{22} = +21.9°$ (tetrachloroethane) (c = 4.35)	$[\alpha]_D^{25} = +9.5°$ (tetrachloroethane) (c = 2.99)
	Yield: 97.1%	Yield: (partially purified), 95%	m.p. 73° C. (b)	

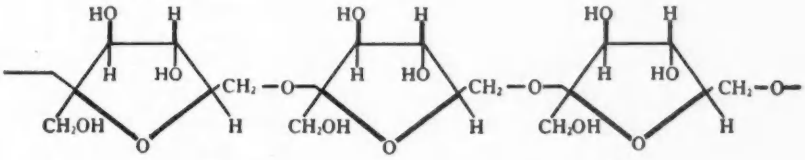
nature of the final product (trimethyl levan) when, in the first stage of the methylation, potassium hydroxide was substituted for sodium hydroxide. However, apparently a purer product is obtained when the later methylation with methyl iodide and silver oxide is carried out in two stages, using a smaller quantity of methyl iodide and silver oxide, rather than in one operation in which a large excess of methyl iodide is necessarily employed.

The trimethyl levan obtained in this way, when hydrolyzed with dilute sulphuric acid, yielded the same crystalline 1:3:4 trimethyl fructofuranose obtained previously by Hibbert, Tipson and Brauns (7) from the levan synthesized by the action of *B. mesentericus* on sucrose. A melting-point determination of a mixture of the two trimethyl fructofuranoses showed no alteration in value.

The triacetyl levan was also identical with that prepared from the levan synthesized by the action of *B. mesentericus*. A point of some importance in the preparation and isolation of the pure crystalline 1:3:4 trimethyl fructofuranose is that the barium carbonate used for neutralizing the hydrolytic agent (sulphuric acid) should be quite free from traces of free alkali, since, in presence of the latter, the very reactive trimethyl fructofuranose readily undergoes chemical change.

Hydrolysis of the highly-purified levan gave a yield of 99% pure, snow-white crystalline fructose. It was found advisable to employ oxalic acid rather than mineral acids for the hydrolysis, as in this way all tendency towards side-reactions was avoided.

The above facts establish the identity of the levan synthesized from sucrose by *B. subtilis* and *B. mesentericus* respectively, as a polymerized 2:6 anhydro fructofuranose (7, p. 228).



Experimental

Preparation of Levan by the Action of B. subtilis on Sucrose

The levan was prepared by Mr. H. L. A. Tarr using a technique slightly modified from that employed in the previous investigation with *B. mesentericus* (4). The following solutions were prepared: (a) sucrose in distilled water, 20%; (b) peptone, 0.2%; Na_2HPO_4, 0.4%; and KCl, 1.0%, in distilled water. Both solutions were sterilized separately in 500-cc. portions in one-litre Erlenmeyer flasks in an autoclave for 20 min. at 15 lb. steam pressure. The resulting solutions were cooled, mixed carefully, employing aseptic technique, inoculated with one 2-mm. loopful of a 48-hour-old sucrose-broth culture of *Bacillus subtilis* and incubated for six days at 37.5° C.

Isolation of the Levan

Two litres of this solution are evaporated to a syrup (about 350 cc.) under reduced pressure (12 mm.). The syrup is then poured drop by drop, at room temperature, into three litres of methyl alcohol (96-98%), the solution being agitated vigorously throughout the addition. The precipitated levan is generally obtained as a friable, white mass but occasionally separates out as a sticky, white product. In either case it is filtered off, mixed with about 500 cc. of pure methyl alcohol (96-98%) and a quantity of glass beads, and shaken for about 12 hr. in the shaking machine. It is then obtained as a white, friable powder which is decanted from the beads, filtered and washed with absolute ethyl alcohol, followed by anhydrous ether. After drying for 24 hr. over calcium chloride in a vacuum desiccator, the levan is obtained as a white friable powder with an ash-content of 0.8-1.0%. It reduces Fehling's solution only very slightly. Yield 55-60 gm., or 60-65% calculated on the fructose portion present in the sucrose taken. In order to remove the inorganic salts the levan (60 gm.) is dissolved in 500 cc. of cold distilled water, 5 cc. of 10% aqueous ammonia solution added, and the viscous solution electrodialyzed for 72 hr. during which pure distilled water is circulated through the outer cell compartments*. A small amount of a slimy precipitate consisting of the bacterial cells settles out in the levan solution and this is separated by centrifuging for about 20 min. at around 3,000 r.p.m.**. The clear (opalescent) solution is then again evaporated under diminished pressure to about 500 cc. and the levan re-precipitated and isolated as before. So obtained, it is a snow-white amorphous powder, which does not reduce Fehling's solution and is completely free from ash.

It is slightly soluble in distilled water, giving an opalescent solution. It is also soluble in glycol and glycerol but insoluble in methyl and ethyl alcohol. Prior to analysis the substance was dried at 100° C. over phosphorus pentoxide for 24 hr. at 0.2 mm. pressure. Analysis: 0.0207 gm. gave: H, 6.16; C, 44.4%. Calcd. for $(C_6H_{10}O_5)_x$: H, 6.2; C, 44.42%. Rotation: (a) Wt. taken 0.1544 gm. $[\alpha]_D^{22} = -46.1°$ (in water), c = 5.88. (b) Wt. taken 0.1002 gm. $[\alpha]_D^{21} = -45.7°$ (in water), c = 5.14. ((a) and (b) were two different preparations).

Hydrolysis of the Pure Levan

Anhydrous purified levan (5 gm.) was dissolved in 50 cc. of cold distilled water and 0.25 gm. of anhydrous oxalic acid added. The mixture was heated in the water bath for one hour, the solution cooled, neutralized by shaking with pure precipitated calcium carbonate, filtered, the residue washed thoroughly with hot distilled water and filtrate and washings evaporated under reduced pressure. The glass-like residue was extracted with 60 cc. of absolute ethyl alcohol, filtered from a small amount of calcium salts, and the filtrate allowed

**The ammonia solution is added in order to prevent the formation of "acid spots" or "acid layers" in the solution during the electrodialysis.*

***With other bacterial products it was found better to remove most of the cells by centrifuging the original solution for about 20 min. at 3000 r.p.m.*

to evaporate spontaneously over calcium chloride under reduced pressure. The syrupy residue, on inoculation with crystalline fructose, crystallized to a solid mass. This was purified by dissolving in 50 cc. of distilled water, adding a small amount of decolorizing carbon, boiling for two minutes, filtering and again concentrating under reduced pressure. The thick syrup on mixing with absolute ethyl alcohol crystallized completely. It was dried at room temperature for two days (to constant weight) over phosphorus pentoxide at 0.5 mm. Yield, 5.5 gm. (99% of snow-white crystalline fructose). $[\alpha]_D^{22} = -90.5°$ (in water), c = 4.8.

Preparation of Trimethyl Levan

The essential factor for "ease of methylation" and production of a fully methylated levan is the use of a pure, *ash-free* levan. With an ash content of only 0.5% it was found that the methylated product obtained by the action of dimethyl sulphate and sodium hydroxide on levan was insoluble in either chloroform or methyl iodide, and could not be methylated further by Purdie's method (silver oxide and methyl iodide).

Method (a). Using dimethyl sulphate and sodium hydroxide. Pure, electrodialyzed, ash-free levan (10 gm.) was dissolved in 10 cc. of cold distilled water in a 250-cc. three-neck flask and methylated using 45 cc. of dimethyl sulphate and 110 cc. of 35% sodium hydroxide. Each of these reagents was added in ten equal portions as follows, the mixture being vigorously agitated throughout the entire period.

TABLE II

ADDITION OF REAGENTS DURING FIRST METHYLATION OF LEVAN

No. of addition	1	2	3	4	5	6	7	8	9	10
Time from commencement, hr.	0.0	1.0	2.0	2.5	3.0	3.5	4.0	4.25	4.5	4.75
Temperature, ° C.	20	22	38	46	50	55	68	70	70	70

After the fourth addition (temperature, 46° C.) a white, syrupy product commenced to separate out. After the tenth addition the temperature was raised to 100° C. and maintained there for one hour, the product being vigorously agitated throughout this period. The methylated levan separated out on the surface in the form of a tough, light yellowish colored cake. The hot mother liquor was carefully decanted off, cooled and extracted three times by shaking with chloroform. The united chloroform extracts were evaporated

TABLE III

ADDITION OF REAGENTS DURING RE-METHYLATION OF LEVAN

No. of addition	1	2	3	4	5	6	7	8	9	10
Time from commencement, hr.	0.0	0.25	0.50	0.75	1.0	1.25	1.50	1.75	2.0	2.25
Temperature, ° C.	52	58	61	61	65	66	67	70	70	72

to a syrup under diminished pressure at 30° C., and this then added to the "cake" in the methylating flask and the whole re-methylated employing vigorous agitation and using the same amounts of dimethyl sulphate and sodium hydroxide as employed in the first methylation.

The mixture was now heated at 100° C. for one hour and stirred vigorously throughout this period. It was allowed to cool and on standing for a short time the solid methylated levan settled out along with the sodium sulphate. The liquid was separated from the powdery solids by centrifuging, and then extracted with chloroform as before. The remaining mixture of solid methylated levan and sodium sulphate was dried at 50° C. in a vacuum oven and then extracted three times under reflux with boiling chloroform. The united chloroform extracts were dried over anhydrous magnesium sulphate, filtered, and the chloroform removed by evaporation from a three-neck flask under reduced pressure.

The thick syrup which was left was then methylated with methyl iodide and silver oxide. In order to prevent undesirable side-reactions a slight modification of the usual method as introduced by Hibbert and coworkers (5, 6) was employed whereby the silver oxide was added in small portions and kept in suspension by vigorous mechanical stirring. The syrupy methylated product was dissolved in 100 gm. of methyl iodide and 68 gm. of silver oxide (freshly prepared and previously carefully dried for 24 hr. at 50° C./20 mm. and one hour at 78° C./0.2 mm.) added in ten portions, a new addition being made every 30 min. at a temperature of 47° C. After the last addition of the oxide, the mixture was agitated for an additional hour at the same temperature, the stirrer being so adjusted as to prevent the formation of a layer of silver oxide at the bottom of the flask. Under these conditions very efficient methylation took place. The product was cooled, chloroform added, the mixture agitated for 15 min. then centrifuged, and the residual silver salts extracted three times, under reflux, with boiling chloroform. The united chloroform extracts were colored brown due to the presence of a small amount of a colloidal silver salt. The latter was removed by adding 10 cc. of concentrated aqueous ammonia solution to the chloroform solution and shaking for three to four hours. The mixture was then filtered to remove the pasty silver salt. The clear chloroform filtrate (or light emulsion) was then dried with magnesium sulphate, filtered, and the filtrate evaporated under diminished pressure at 30° C., leaving the trimethyl levan as a pale yellow, glass-like solid.

Purification of the Trimethyl Levan

This was carried out by dissolving the product in boiling methyl acetate and then pouring the cooled solution into ligroin (b.p. 30-50° C.) with vigorous stirring. The trimethyl levan separated out as a fine white powder. It was filtered off, digested with boiling ether for half an hour, the cold ether solution centrifuged, the product washed with ligroin and dried over sulphuric acid under reduced pressure. It was then further dried at 78° C. over phosphorus pentoxide under a pressure of 0.2 mm. M.p. 145° C., yield, 75%. Analysis:

0.0199 gm. gave: H, 7.6; C, 52.77%. 0.0207 gm. gave 0.0703 AgI; OCH_3, 45.9%. Calcd. for $(C_9H_{16}O_5)_x$; H, 7.9% C, 52.9%; OCH_3, 45.6%. $[\alpha]_D^{21} = -88.0°$ (in tetrachloroethane), c = 3.57.

Method (b). Using dimethyl sulphate and potassium hydroxide. Pure, dry, electrodialyzed ash-free levan (20 gm.) was dissolved in 20 cc. of distilled water and methylated, in the same manner as in the previous experiment, with 90 cc. of dimethyl sulphate and 225 cc. of 30% potassium hydroxide. The methylated product so obtained was then re-methylated twice by Purdie's method, using each time 150 gm. of methyl iodide and 80 gm. of pure dry silver oxide. The resulting yellowish syrup (25 gm.), obtained by extraction with chloroform, was boiled with dry ether whereby the trimethyl levan was obtained as a light yellowish powder. Yield 88.0%. The purification of the product was carried out by the method already outlined and yielded a pure, white powder, m.p. 145-146° C. Analysis: 0.0217 gm. gave: H, 8.14; C, 52.8%. 0.0218 gm. gave 0.0732 gm. AgI; OCH_3, 44.3%. Calcd. for $(C_9H_{16}O_5)_x$: H, 7.9; C, 52.77; OCH_3, 45.6%. $[\alpha]_D^{22} = -87.2$ (in tetrachloroethane), c = 4.77.

Hydrolysis of the Trimethyl Levan and Isolation of Pure 1:3:4 Crystalline Trimethyl Fructofuranose

Trimethyl levan (15 gm.) was dissolved in 75 cc. of 96% ethyl alcohol, and then 5 cc. of *N*/1 H_2SO_4 and 100 cc. of distilled water added. The solution was heated on the water bath (95-100° C.) for about six hours, and then 75 cc. of the mixture distilled off and 75 cc. of distilled water added. The product was heated for 12 hr. at the same temperature (95-100° C.), a further 75 cc. distilled off under diminished pressure, and the same amount (75 cc.) of distilled water again added. Working in this way the presence of any fructofuranoside was avoided. A determination of the optical rotation was then made and, after heating the solution for a further six hours at 95-100° C., a second measurement was taken. A constant value was found. The slightly yellow solution was then heated at 96° C. for a short time with decolorizing carbon, filtered, cooled, neutralized by shaking with precipitated barium carbonate (free from hydroxide) and then shaken for 12 hr. with decolorizing carbon. The solution was filtered, the residue washed thoroughly with hot water and the united clear, colorless filtrate and washings evaporated to a syrupy consistency under reduced pressure (12 mm.) at 30° C. On standing for 12-15 hr. the thick syrup solidified completely to a crystalline cake. It was dissolved in pure, dry ether, the solution filtered from traces of barium salts, and the ether removed under reduced pressure. The residue which remained crystallized on standing over night to a light yellow crystalline cake. Yield, 16.1 gm. (98.5%). It was crystallized from ligroin and then recrystallized from carbon tetrachloride, m.p. 74° C. A mixed melting-point determination carried out with a mixture of this material and the 1:3:4 trimethyl fructofuranose prepared from the levan synthesized by the action of *B. mesentericus* on sucrose gave the same value. Found $[\alpha]_D^{22} = +21.55$ (c = 3.56); solvent,

tetrachloroethane. Corresponding rotation of the 1:3:4 trimethyl fructose from *B. mesentericus*-levan at the same temperature and using the same solvent was +21.9°.

Preparation of Levan Acetate

Pure levan (4.5 gm.) was added to a mixture of 25 cc. of pure dry pyridine and 18 cc. of pure acetic anhydride and the combined products boiled for about five minutes. The levan went into solution and simultaneously a gel was formed, which was not dissolved by the addition of a further 25 cc. of pyridine. The gelatinous mass was poured into 400 cc. of finely powdered ice and water, whereupon the levan acetate precipitated out as a fine, snow-white powder. It was filtered off, washed thoroughly with distilled water and dried in a vacuum desiccator over phosphorus pentoxide and solid sodium hydroxide. Yield, 7.0 gm. (87%). Further purification was effected by dissolving the product (7.0 gm.) in methyl acetate, filtering through a layer of Fuller's earth and then re-precipitating the acetate by pouring the solution into 100 cc. of dry ether. After standing overnight at 0° C., the mixture was centrifuged, washed with pure, dry ether, again centrifuged, washed with ligroin (b.p. 30-50° C.), filtered with suction and dried over sulphuric acid under reduced pressure. So obtained, triacetyl levan is a very fine snow-white powder, soluble in chloroform and tetrachloroethane with formation of clear, transparent, viscous solutions. When heated it commences to sinter at 103° C. but is only completely melted at 133° C. Analysis: Saponification: 0.1166 gm. required 12.3 cc. *N*/10 NaOH. Found: H, 5.8; C, 49.97; —$COCH_3$, 45.5%. Calcd. for $(C_{12}H_{16}O_8)_n$: H, 5.6; C, 49.98; —$COCH_3$, 44.7. $[\alpha]_D^{22} = +9.53°$; tetrachloroethane, (c = 4.58); another preparation gave $[\alpha]_D^{24} = +9.0$ (c = 1.96).

Acknowledgment

The authors wish to express their best thanks to Mr. H. L. A. Tarr for his kindness in preparing for them the large amount of levan used in this investigation.

References

1. BERGEY, D. H. Manual of determinative bacteriology, Williams and Wilkins, Baltimore, 1930.
2. FREUDENBERG, K. and BRAUN, E. Ann. 460: 288-304. 1928.
3. FREUDENBERG, K. and coworkers. Ber. 63: 1962. 1930 (footnote).
4. HARRISON, F. C., TARR, H. L. A. and HIBBERT, H. Can. J. Research, 3: 449-463. 1930.
5. HIBBERT, H. and CARTER, N. M. J. Am. Chem. Soc. 50: 3120-3127. 1928.
6. HIBBERT, H. and MORAZAIN, J. G. Can. J. Research, 2: 214-217. 1930.
7. HIBBERT, H., TIPSON, R. S. and BRAUNS, F. Can. J. Research, 4: 221-239. 1931.
8. HAWORTH, W. N., HIRST, E. L. and THOMAS, H. A. J. Chem. Soc. 821-824. 1931.
9. JACKSON, R. F. and GOERGEN, S. M. Bur. Standards J. Research, 3: 27-38. 1929.
10. PAULI, W. Biochem. Z., 152: 355-359. 1924.

OXIDATION-REDUCTION POTENTIALS IN CULTURES OF ES. COLI[1]

By Eldon M. Boyd[2] and Guilford B. Reed[3]

Abstract

Following the evidences advanced in a previous paper that metabolic gases are concerned in the production of growth-oxidation-reduction potentials observed in cultures of bacteria, experiments have been arranged to test the effects of such gases in comparison with other factors. Growth and oxidation-reduction potentials have been determined simultaneously in anaërobic buffered broth cultures of *Es. coli*. It is shown that in sugar-free broth there is a sudden fall in potential at the beginning of the logarithmic growth period. The addition of glucose to the medium was found to induce an earlier and more precipitous fall in potential which is coincident with the first evolution of gas. At the same time there was found to be evidence of a non-gaseous electromotively active system in these cultures.

Introduction

In a former paper (3) it was suggested from a study of gas-metal electrode potentials in sterile culture media that metabolic gases, particularly hydrogen, produced by the growth of bacteria are responsible for at least a part of the mechanism of observed oxidation-reduction potentials at noble metal electrodes in such cultures. This conclusion is to a considerable degree at variance with explanations offered by most observers of oxidation-reduction potentials of growing cultures of bacteria, as for example the work of Gillespie (10) with soil organisms, Cannan, Cohen and Clark (4) with cultures in milk, Aubel and Genevois (2) with *B. coli* in glucose broth, Thornton and Hastings (18), Harvey (11), Coulter and Isaacs, (7) with broth cultures of typhoid bacilli, Aubel, Aubertin and Genevois (1) with anaërobes, Hewitt (12, 13, 14, 15) with *Streptococci*, *B. diphtheriae*, *Staphylococci* and *Pneumococci*. Experiments have accordingly been designed to investigate the effect of gases as observed in the former paper, in comparison with other factors in the mechanism of the production of growth-oxidation-reduction potentials in cultures of *Es. coli*.

Time-potential-growth curves

Beef-extract broth was freshly prepared in the usual manner except that 35 gm. of disodium phosphate (0.1*M*) was added in place of sodium chloride and the pH adjusted with hydrochloric acid to pH 7.3. This was dispensed into one-litre florence flasks so that the level of the medium was brought into the neck. The flasks were then autoclaved, two sterile bright platinum electrodes and a sterile saturated potassium chloride-agar bridge quickly inserted and the surface covered with sterile vaseline. The assembled flasks of media were then held overnight in an incubator at 37.5° C. This process assured sterility and also allowed sufficient time for the electrodes to come to

[1] *Manuscript received April 25, 1931.*
Contribution from the Department of Bacteriology, Queen's University, Kingston, Ontario, Canada.

[2] *Graduate student, Queen's University.*
[3] *Professor of Bacteriology, Queen's University.*

equilibrium. The flasks were inoculated by inserting a loopful of an 18-hr. broth culture of *Es. coli* through a temporary hole made in the seal. Incubation was continued at 37.5° C. At the same time, the flasks were connected with a saturated calomel electrode and the e.m.f. observed approximately every hour. The readings are indicated in Table I. From time to time, after thoroughly rotating the flasks to assure homogeneity, 1-cc. samples were withdrawn and plate counts made of the number of bacteria present. Quadruplicate plates were made at each determination. Control flasks were similarly arranged and maintained, without inoculation, at 37.5° C. and e.m.f. readings were made simultaneously with those in the inoculated flasks.

TABLE I

THE RELATION OF THE *Eh* OF AN ANAËROBIC BUFFERED BROTH CULTURE OF *Es. coli* TO TIME, AND GROWTH RATE OF THE ORGANISM

Time, hr.	Oxidation-reduction potential, *Eh*						Plate count, organisms per cc.
	Sterile control			Inoculated flask			
	Electrode *A* volts	Electrode *B* volts	Mean *Eh* of control volts	Electrode *A* volts	Electrode *B* volts	Mean *Eh* of inoculated volts	
0	+0.148	+0.087	+0.118	+0.085	+0.020	+0.053	
0.5	+0.147	+0.075	+0.111	+0.087	+0.014	+0.051	
1	+0.155	+0.072	+0.114	+0.090	+0.025	+0.058	510
2	+0.145	+0.102	+0.124	+0.090	+0.025	+0.058	156×10
2.5	+0.150	+0.129	+0.140	+0.097	+0.012	+0.055	408×10
3	+0.152	+0.123	+0.138	+0.094	+0.007	+0.051	183×10^2
3.5	+0.151	+0.125	+0.138	+0.038	−0.007	+0.016	
4	+0.152	+0.124	+0.138	−0.006	−0.007	−0.007	
5	+0.153	+0.123	+0.138	−0.039	−0.053	−0.046	119×10^3
6	+0.148	+0.120	+0.134	−0.060	−0.080	−0.070	478×10^3
7	+0.147	+0.120	+0.134	−0.048	−0.050	−0.049	131×10^4
8	+0.148	+0.112	+0.130	−0.091	−0.070	−0.081	109×10^5
9	+0.146	+0.111	+0.129	−0.075	−0.063	−0.069	110×10^5
10	+0.143	+0.112	+0.128	−0.078	−0.092	−0.085	224×10^5
12	+0.138	+0.118	+0.128	−0.126	−0.120	−0.123	541×10^5
14	+0.138	+0.113	+0.126	−0.105	−0.144	−0.125	454×10^5
15	+0.137	+0.110	+0.124	−0.112	−0.156	−0.134	687×10^5
16	+0.137	+0.115	+0.126	−0.118	−0.155	−0.137	798×10^5
24	+0.130	+0.120	+0.125	−0.175	−0.250	−0.213	570×10^5
36	+0.080	+0.100	+0.090	−0.190	−0.298	−0.244	
50	+0.020	+0.060	+0.040	−0.208	−0.340	−0.274	375×10^5
100	−0.030	+0.020	−0.005	−0.200	−0.315	−0.258	195×10^5
125	−0.071	−0.031	−0.051	−0.160	−0.240	−0.200	370×10^5
200	−0.104	−0.078	−0.091	−0.150	−0.210	−0.180	

The results of this experiment are tabulated in Table I and the means of the oxidation-reduction potential readings and dilution plate counts for the first 30 hr. are plotted in Fig. I. It will be noted from these data that the sterile medium underwent little change in potential from the initial value reached after standing overnight. The slight reducing drift which set in at 24 to 36 hr. reached a maximum at about 200 hr. and represents a fall of

approximately 0.2 volts to a low level of −0.091 volts. Further readings not shown in the table or graph indicated that the potential remained approximately constant at this level for the several succeeding days. The initial slight rise in potential at four to six hours, shown in the table and figure, has been a common finding in various samples of broth. It apparently bears no relation to the anaërobic aging of the medium as freshly autoclaved broth and broth which had stood for a week under a vaseline seal have both given a similar rise. The slight polarization currents passing through the medium while e.m.f. readings were being observed may be responsible. Knight (16) using a valve potentiometer obtained positive drifts of one millivolt per three minutes in sterile broth when the electrode system was kept closed; with pin electrodes the drift was greater, five millivolts per minute.

In the inoculated flasks the potential remained constant, except for the slight polarization rise, for some three hours or until the organisms reached 10000 to 20000 per cc., Table I and Fig. 1. During the logarithmic phase of growth, from two to nine hours, the numbers increased to some 11 million per cc. and the potential fell to *E*h −0.075 volts. At about this point there was a definite change in the rate of fall in potential to a more gradual drift which continued until at 50 hr. an *E*h of −0.274 volts was reached.

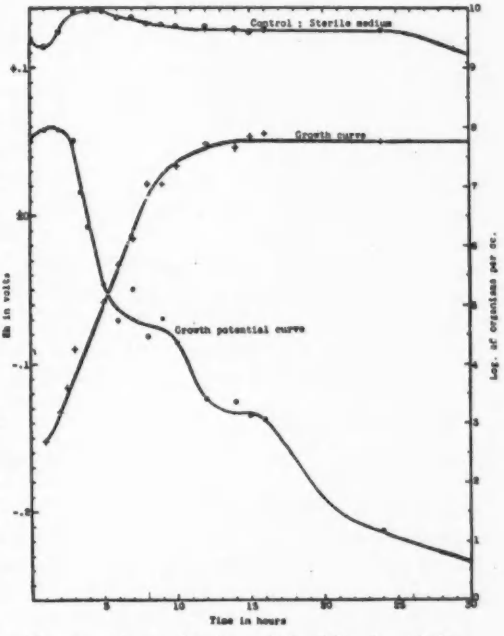


FIG. 1. *Curves showing relationship of oxidation-reduction potentials to growth rate of Es. coli in buffered broth.*

This change in rate (Fig. 1) in the negative drift has been observed in many but not all samples of broth. It will be noted below that it does not occur if glucose is added to the medium. It may therefore be due to the relative absence of carbohydrates in the broth in which, as ordinarily prepared, no precaution is taken to ferment out the sugars. Since the change in rate occurs at approximately the reduction level attained by sterile broth under anaërobic conditions produced either by long standing (3) or by "deaëration" with oxygen-free nitrogen (5, 16), it appears to be a reasonable inference that the initial metabolism of *Es. coli* under "anaërobic" conditions is a utilization of oxidizing substances present in freshly autoclaved broth. Whether or not this is due simply to oxygen, as suggested by Coulter (5), or whether other

oxidizing substances are involved is not apparent. It is evident that at this point, however, a different type of metabolism sets in, a true anaërobic metabolism. This is characterized potentiometrically by the formation of substances of a greater reducing intensity. It is probable that at least one of these in the case of *Es. coli* is nascent hydrogen or hydrogen gas (3). Reduced sulphydryl groups may also enter into the mechanism. That active growth should cease at this point may be either the cause or the effect of the potential changes noted; in any case it appears to be significant.

The Effect of Glucose

To a four-litre sample of broth prepared as before, 1% of glucose was added, the medium autoclaved and set up in a potentiometer circuit with two sterile bright platinum electrodes as in the former experiments. After standing overnight it was inoculated from an 18-hr broth culture of *Es. coli* and potential readings begun. A gas tube was inserted just below the vaseline seal, the gas collected and measured as it formed. The gas produced was expressed as cc. per minute per litre of culture.

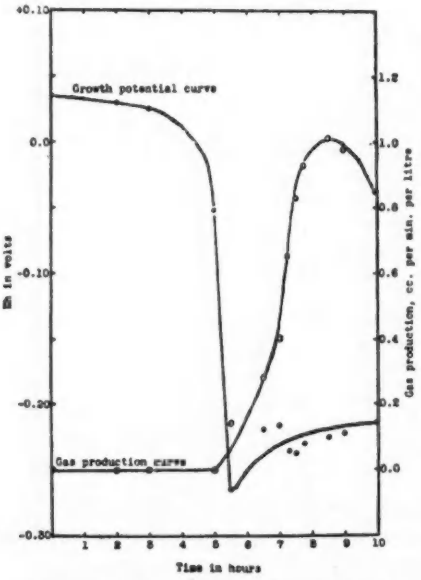


FIG. 2. *Curves showing the relationship of oxidation-reduction potentials to the rate of gas production in cultures of Es. coli in glucose broth.*

As shown in Table II and Fig. 2 the potential remained approximately stationary for about four hours, as in the former experiments, and then assumed a gradual negative drift followed at five to five and one-half hours by a more abrupt fall to an *E*h value of −0.265 volts. The pH of the cultures remained approximately constant at the initial pH 7.4 for several hours due to the 0.1 *M* phosphate present, then became slightly more acid and stationary again at pH 6.5 during the period of increasing positive potential. The potential figures in the tables and graphs are corrected for pH changes.

The potential curves of *Es. coli* in glucose broth, Fig. 2, differ from those generally observed in similar broth without added glucose, Fig. 1, in that an abrupt change in the rate of negative fall in potential is absent. This further suggests, as mentioned in the previous section, that the primary "aërobic" metabolism passes gradually to "anaërobic" when glucose is present. Moreover since the addition of 1% glucose to the media caused the cultures to reach their negative *E*h limit in five and one-half hours as against 25-50 hr. with various samples of broth without added glucose, it seems not unreasonable to suppose that the variation in different

samples of broth in this respect is probably due to the relative amount of glucose or other fermentable sugar present.

TABLE II

THE RELATION OF POTENTIAL CHANGES TO GAS PRODUCTION IN BUFFERED GLUCOSE-BROTH CULTURES OF *Es. coli*

Time, hr.	Oxidation reduction potential, *Eh*			Gas production, cc. per min. per litre
	Electrode *A* volts	Electrode *B* volts	Mean *Eh* volts	
0	−0.006	+0.080	+0.036	0
2	−0.008	+0.067	+0.030	0
3	−0.014	+0.064	+0.025	0
5	−0.102	−0.003	−0.052	0
5½	−0.276	−0.255	−0.265	0.14
6½	−0.205	−0.233	−0.219	0.28
7	−0.203	−0.230	−0.217	0.40
7¼	−0.221	−0.251	−0.236	0.65
7½	−0.226	−0.249	−0.238	0.83
7¾	−0.220	−0.240	−0.230	0.93
8½	−0.213	−0.237	−0.225	1.11
9	−0.210	−0.236	−0.223	0.98
10	−0.200	−0.233	−0.217	0.85

The production of gas from the glucose-broth cultures, as shown in Table II and Fig. 2, began about five hours after inoculation and very rapidly increased in rate, though the maximum production of 1.14 cc. per litre of culture was not reached until some three hours after the beginning of the evolution. The sudden drop in the potential, previously noted and as indicated in Fig. 2, was observed to be coincident with the appearance of the first bubbles of gas. That the reducing limit of *Eh* and the commencement of gas evolution should be attained at the same time again points to the probable significance of gases in the mechanism of growth reduction intensities.

A Non-gaseous System

A sample of glucose broth was set up as in the last experiment, inoculated with *Es. coli* and allowed to incubate for two weeks until gas production had ceased. A vigorous stream of nitrogen was then passed through the culture to drive out the remaining metabolic gases. E.m.f. readings were secured before and during this process which was continued until the *Eh* reached an equilibrium value. It was found that the time required for the establishment of potential equilibrium under these circumstances varied as the age of the culture and the rate of flow of the nitrogen. When potential stability was reached the stream of nitrogen was turned off and further *Eh* values recorded until again an equilibrium value was reached. The pH of the fluid was 6.6. The results of the procedure are shown in Table III. It will be observed that under these conditions nitrogen had little effect on the *Eh* of an old culture.

There was a drop in potential from −0.261 to −0.236 volts just after admitting the gas but an equilibrium value of −0.245 volts was established in half an hour and remained steady for a further hour. On turning off the gas the potential rose to −0.257 volts or approximately the *E*h of the culture before nitrogen was admitted. It may therefore be concluded that any gases remaining dissolved in the medium after two weeks incubation have no effect upon *E*h. In consequence the reduction potential indicated by the culture must depend upon some non-gaseous electromotively active system whose reducing range at least reaches *E*h −0.260 volts.

TABLE III

THE EFFECT ON THE OXIDATION-REDUCTION POTENTIAL OF PASSING NITROGEN THROUGH A TWO-WEEKS-OLD GLUCOSE-BROTH CULTURE OF *Es. coli* AT PH 6.6

Duration of nitrogen action, min.	Oxidation-reduction potential, *E*h		
	Electrode *A* volts	Electrode *B* volts	Mean *E*h volts
0	−0.260	−0.262	−0.261
5	−0.242	−0.230	−0.236
10	−0.240	−0.230	−0.235
30	−0.246	−0.244	−0.245
60	−0.246	−0.248	−0.247
90	−0.248	−0.248	−0.248
Gas off			
5	−0.255	−0.253	−0.254
15	−0.260	−0.254	−0.257

In order to ascertain the range of this system, air was forced through the culture until e.m.f. readings indicated equilibrium. The gas stream was then turned off and e.m.f. equilibrium again determined in order to eliminate the effects of stirring. The results shown in Table IV indicate that air causes a gradual increase in potential to approximately that of the original sterile broth. It was demonstrated in a previous paper (3) that the effect of air on broth potentials is produced slowly. The dissolved air was then removed with a stream of nitrogen which caused a fall in potential to about −0.2 volts. Potentiometrically this may be considered to have caused the air-broth potential with platinum to be replaced by the potential of the oxidized non-gaseous system. The value reached may therefore be considered the *E*h of the non-gaseous system. Other similar experiments yielded the values *E*h −0.209 and −0.215 volts. From these experiments it may therefore be concluded that in two-weeks-old broth cultures of *Es. coli* there is present a non-gaseous oxidation-reduction system with an *E*h range of −0.210 to −0.260 volts. It is not suggested, however, that these values represent the extreme limits of this system. The system was found to be reversible, for on incubating the oxidized

system (*Eh*, −0.210 volts) overnight the potential fell to −0.248 volts. A stream of hydrogen passed into the oxidized system (*Eh*, −0.210 volts) caused a negative drift to −0.293 volts but on washing out the hydrogen with nitrogen the potential returned to −0.214 volts. Hence it may be concluded that gaseous hydrogen does not reduce the system but superimposes at platinum electrodes a more reducing *Eh*.

TABLE IV

THE EFFECT OF AIR ON THE OXIDATION-REDUCTION POTENTIAL OF A TWO-WEEKS-OLD GLUCOSE-BROTH CULTURE OF *Es coli*

Duration of air admission, min.	Oxidation-reduction potential, *Eh*		
	Electrode *A* volts	Electrode *B* volts	Mean *Eh* volts
0	−0.260	−0.254	−0.257
5	−0.158	−0.140	−0.149
60	+0.042	+0.054	+0.048
70	+0.075	+0.091	+0.083
80	+0.074	+0.092	+0.083
Air off			
5	+0.094	+0.110	+0.102
10	+0.085	+0.107	+0.096
Air removed with nitrogen			
5	−0.129	−0.133	−0.131
20	−0.172	−0.182	−0.177
30	−0.170	−0.180	−0.175
Nitrogen off			
15	−0.185	−0.221	−0.203
20	−0.182	−0.224	−0.203

Discussion

It has been shown that when bright platinum electrodes are used, cultures of *Es. coli* under anaërobic conditions attain an *Eh* of −0.28 volts in buffered broth and −0.265 volts in buffered glucose broth. This is comparable with Cannan, Cohen, and Clark's (4) observation of −0.3 volts as the lowest limit of *Es. coli* cultures in milk and with numerous similar studies. The sterile buffered broth under similar conditions was found to reach equilibrium values of -0.091 ± 0.015 volts. With gold electrodes Coulter (5, 6) reported values varying from −0.185 to −0.135 volts, while Knight (16) found *Eh* −0.130 volts. Using Clark's indicators, Dubos (8) found an *Eh* of −0.18 volts at pH 7.8; Coulter (6) −0.135 to −0.150 volts at pH 7.2-7.5; and Knight (16) −0.150 at pH 7.45.

Data have been presented in the previous sections which indicate that growth and gas production are correlated with potential changes. The initial drop in potential occurs simultaneously with the beginning of logarithmic growth. In glucose-broth cultures the appearance of gas was also coincident with the reducing drift in potential. This further substantiates the authors' previous suggestion (3) that hydrogen is a factor in potential changes in growing cultures of *Es. coli* and is in agreement with Lepper and Martin's (17) observations that hydrogen and sulphides are produced by organisms which induce highly reducing potentials. It was also shown the addition of glucose caused a more precipitous fall in potential to the negative limiting value than occurred in broth without glucose. Dubos (9) found that reduction of indicator dyes by washed cells of *Pneumococcus* occurs only if glucose is present.

It has at the same time been shown that there is present in broth cultures of *Es. coli* a non-gaseous reversible oxidation-reduction system active between the limits of *E*h -0.210 and -0.280 volts at pH 6.6. This system is reversibly oxidized by air and reduced in broth culture but is not reduced by gaseous hydrogen.

The mechanism of broth potentials of *Es. coli* cultures from the present investigation may be tentatively summarized as follows: A preliminary rise may occur due probably to polarization currents. As growth begins an "aërobic" metabolism ensues for a varying time during which the organisms utilize oxygen or other oxidizing agents present in broth. This is followed by a true "anaërobic" metabolism during which the less oxidizing or slightly reducing products (*E*h, -0.100 volts) are oxidized producing a highly reducing system electromotively active between the limits of -0.210 and -0.280 volts. As gas forms, a still more negative potential may develop at platinum electrodes.

Summary

1. Growth and oxidation-reduction potentials have been simultaneously studied in anaërobic buffered broth cultures of *Es. coli*.

2. It has been shown that in sugar-free broth there is a sudden fall in potential at the beginning of the logarithmic growth period.

3. The addition of glucose to the media was found to induce an earlier and more precipitous fall in potential. It was shown that the fall in potential was coincident with the first evolution of gas.

4. Evidence has been advanced to indicate that in addition to the gaseous there is also a non-gaseous electromotively active system with an *E*h range of -0.210 to -0.280 volts in cultures of *Es. coli*.

References

1. AUBEL, E., AUBERTIN, E. and GENEVOIS, L. Ann. physiol. physicochim. biol. 5: 1-11. 1929.
2. AUBEL, E. and GENEVOIS, L. Compt. rend. 184: 1676-1678. 1927.
3. BOYD, E. M. and REED, G. B. Can. J. Research, 4: 54-68. 1931.
4. CANNAN, R. K., COHEN, B. and CLARK, W. M. U.S. Public Health Report, Supp. 55. 1926.

5. COULTER, C. B. J. Gen. Physiol. 12: 139-146. 1928.
6. COULTER, C. B. Proc. Soc. Exptl. Biol. Med. 27: 397-398. 1930.
7. COULTER, C. B. and ISAACS, M. L. J. Exptl. Med. 49: 711-725. 1929.
8. DUBOS, R. J. Exptl. Med. 49: 507-523. 1929.
9. DUBOS, R. J. Exptl. Med. 50: 143-160. 1929.
10. GILLESPIE, L. J. Soil Science, 9: 199-216. 1920.
11. HARVEY, E. N. J. Gen. Physiol. 13: 13-20. 1929.
12. HEWITT, L. F. Biochem. J. 24: 512-524. 1930.
13. HEWITT, L. F. Biochem. J. 24:667-675. 1930.
14. HEWITT, L. F. Biochem. J. 24: 676-681. 1930.
15. HEWITT, L. F. Biochem. J. 24: 1551-1556. 1930.
16. KNIGHT, B. C. J. G. Biochem. J. 24: 1066-1074. 1930.
17. LEPPER, E. and MARTIN, C. J. Brit. J. Exptl. Path. 11: 137-145. 1930.
18. THORNTON, H. R. and HASTINGS, E. G. J. Bact. 18: 293-318. 1929.

SPECIFIC EFFECT OF MONOCHROMATIC LIGHT UPON PLASMOLYSIS IN PARAMECIUM[1]

By A. H. Hutchinson[2] and M. R. Ashton[3]

Abstract

Investigations conducted during the last three years have shown that light of definite wave-lengths has specific effects upon the growth of organisms, *e.g.*, yeast, paramecium and tomato rot (*Colletotrichum*).

The organism, *Paramecium caudatum*, was irradiated in a quartz cell at room temperature with a Cooper-Hewitt "Lab-arc" for periods of 6 and 24 hr. and the rate of plasmolysis in sodium chloride and sucrose solutions then determined. The organisms passed through the same series of reactions in both solutions but plasmolysis occurred much more rapidly in sodium chloride than in sucrose solutions, even when the latter were of greater concentration.

It would seem that the effect of light is to increase the rate of plasmolysis under all circumstances, although Packard (8) states that the permeability of the cell membrane is increased with increase of division rate in paramecium.

In general there is a marked similarity between the curves showing the increase in rate of plasmolysis and those showing the effect of irradiation on the growth rate of paramecium.

In most cases visible and near ultra-violet light caused an increase in the rate of plasmolysis of less magnitude than that caused by the far ultra-violet. All visible and near ultra-violet lines used, with the exception of 4960 Å, 4078 Å and 3022 Å, caused stimulation of division rate and these lines had the least effect on the rate of plasmolysis.

The single green spectral line ($\lambda = 4960$ Å) had a retarding effect on growth and also caused a decided increase in plasmolysis. The lines 4078 Å and 3022 Å both caused slight decrease in division rate and slight increase in rate of plasmolysis.

It would appear that, with few exceptions, the effect of monochromatic lines of the spectrum in increasing the rate of plasmolysis is greater the shorter the wave-length.

It has been found that in many cases injury to an organism produces rapid increase and stimulation of growth.

From the results of these experiments it would seem that when light energy is absorbed, the physiological condition of the plasma membrane changes. Apparently the conditions accompanying moderate increase in rate of plasmolysis also accompany increase in the rate of cell division in paramecium, and the conditions accompanying excessive increase in rate of plasmolysis result in decrease of growth.

Introduction

Work on the permeability of plant cells under various conditions of illumination points to the fact that within certain limits permeability changes with the amount of illumination.

The effect of light and darkness on the cells of the pulvinus of *Mimosa pudica* was studied by Blackman and Paine (1), who state that light has a marked effect in increasing the permeability of the cells to electrolytes, this effect apparently requiring time to reach a maximum and then falling off. Any sudden change from light to darkness also increases this permeability. The authors believe that the loss of turgor is probably due to the disappearance or inactivation of a considerable part of the osmotic substances of the cells.

[1] *Manuscript received April 9, 1931.*
Contribution from the Department of Botany, University of British Columbia, Vancouver, Canada, with financial assistance from the National Research Council of Canada.

[2] *Professor of Botany, University of British Columbia.*

[3] *Research Assistant.*

J. D. Sayre (9) states that the stomata of *Rumex patientia* do not open when exposed to wave-lengths greater than 690 μ, while the other regions of the visible spectrum, except the violet, seem to be equally effective in causing opening. The limit of effectiveness at the blue end of the spectrum could not be determined from the results of this experiment. Hoagland and Davis (4) showed that the intake of salts by Nitella cells was greater when the cells were illuminated than when they were in darkness. Lepeschkin (6) states definitely that "it is very probable that every kind of protoplasm changes its permeability under the influence of light."

Experiments have also been carried out demonstrating that the permeability of animal protoplasm as well as plant protoplasm varies with the amount of illumination. Packard (8), working on paramecium, showed that the permeability of the cells exposed to light was greater than the controls kept in darkness. This change in permeability was also demonstrated in cells exposed to monochromatic red light, becoming greater as the wave-lengths shortened, and reaching a maximum in the near ultra-violet. He also showed that the permeability of the paramecia varies with the division rate, rapidly dividing cells having a high permeability and those with a slow division rate showing a low permeability.

The investigation described in this paper is a study of the effect of certain monochromatic lines of the mercury arc spectrum on the rate of plasmolysis of *Paramecium caudatum*. From these results suggestions are made as to the effect of ultra-violet light on the permeability of the cell membrane and the osmotic content of the cell. A correlation is made between the effect of ultra-violet light on the growth of paramecium as reported in a previous paper (5) and the effect of ultra-violet light on the plasmolysis of these organisms.

Methods

The source of light was a Cooper-Hewitt "Lab-arc" operated as described in previous papers (5). The organisms, *Paramecia caudatum*, were placed in a quartz cell which fitted into a slot in front of the eye-piece of the Hilger monochromatic illuminator.

It was found possible to obtain continuous division of the paramecia in a culture by the addition of 10-20 cc. of water obtained from a pond adjoining a manure pile, at periods of two or three days.

The culture (2 cc.) was placed in the special quartz receiver at room temperature, and subjected to the irradiation of required wave-length in the slot of the monochromatic illuminator.

The experiments were carried out in two series, one in which sucrose solutions were used as the plasmolyzing agent and the other in which sodium chloride solutions were used.

Part 1

Molar and 0.7 *M* solutions of sucrose were made up for use as plasmolyzing agents. After irradiation for periods of 6 and 24 hr., the organisms were removed from the slot of the illuminator, placed in the sucrose solutions of varying strengths and the rate of plasmolysis noted.

This rate of plasmolysis was obtained in the following way:- a very small drop of the irradiated culture in which one or two specimens of *Paramecium caudatum* were found, was placed on a slide, the capillary pipette of a haemocytometer was then filled to the mark, 1, with sugar solution of the required strength and the drop flooded with the solution. The time required to plasmolyze the organism was recorded by means of a stop watch, the drop being observed under the low power of the microscope.

The same cycle of behavior was noticed with all concentrations, the only variable being the time required for its completion. The organism at first slowed down, frequently changing the plane of its course, then commenced to rotate rapidly, the final stage being reached with complete cessation of movement and ciliary action, apparent thickening of the interior with disappearance of the contractile vacuole, and finally shrinkage, in some cases to half the original size. The end-point was definite since the final stage of plasmolysis, *i.e.*, from the disappearance of the vacuoles to the final shrinkage, took place within two or three seconds.

TABLE I

INCREASE IN RATE OF PLASMOLYSIS EXPRESSED AS PERCENTAGE INCREASE OVER CONTROL RATE

Wave-length	Sodium chloride solutions				Sucrose solutions			
	0.7 Molar		0.3 Molar		Molar		0.7 Molar	
Å	6 hr. %	24 hr. %	6 hr. %	24 hr. %	6 hr. %	24 hr. %	6 hr. %	24 hr. %
6152	30	29	37	41	25	27	31	33
5819–5769	19	21	26	27	13	30	25	41
4960–4916	41	39	33	48	25	25	34	32
4359–4348	20	34	26	33	11	43	23	30
4078–3984	39	39	32	46	19	25	22	38
3821	26	40	35	45	17	18	23	18
3663–3656	28	27	40	25	11	11	7	6
3352–3342	9	11	34	31	23	34	20	32
3132	26	21	37	20	18	21	28	28
3028–3022	44	18	35	24	29	32	33	32
2967	34	36	37	39	28	28	36	37
2804	40	51	34	43	32	36	33	41
2700					32	20	32	22
2054	51	51	58	57				

It was found in a few isolated cases that individual organisms deviated from the average and plasmolyzed either very rapidly or very slowly. These individuals were discarded as abnormal. They may have been at the point of cytolysis or of encystment and consequently did not react normally to osmotic forces.

One paramecium was observed in each drop and the time taken to plasmolyze was noted. The average time taken for plasmolysis was calculated from observations on 10 different paramecia in separate drops of reagent.

The time required by the given solutions to plasmolyze normal, non-irradiated paramecia was ascertained each day, as it was found that there was a slight variation in rate according to the condition of the culture. The effect on the rate of plasmolysis was calculated as the percentage increase or decrease in the irradiated culture as compared with the control.

Part 2

Paramecium caudatum was irradiated for periods of 6 and 24 hr. as previously described, but in place of sugar solutions of varying strengths, sodium chloride solutions (0.7 and 0.3 *M*) were used as plasmolyzing agents. The organisms passed through the same series of reactions as were caused by the sucrose solutions, but it was found that molar sodium chloride plasmolyzed very rapidly as compared with molar sucrose. It was therefore found necessary to use solutions of lower concentration and 0.7 and 0.3 *M* solutions were found satisfactory.

Results

λ 6152 Å

An initial increase in the rate of plasmolysis at the end of the first 6 hr. was followed by a maintenance of this rate for the following 18 hr. in both sucrose and sodium chloride solutions. This region includes two lines of low intensity in the red region of the visible spectrum. Exposure to these rays also resulted in a constant and continued stimulation of growth. Packard (8) found that when paramecia were exposed to light from the red end of the spectrum there was a demonstrable rise in the permeability, with but 0.6% increased intensity of incident light.

λ 5819–5769 Å

This range includes two lines of maximum intensity in the yellow region of the visible spectrum. The effect on the rate of plasmolysis was small as was also the effect on the rate of growth. These rays, even though of maximum intensity, do not seem to exert much effect on the paramecium.

λ 4960–4916 Å

This range includes a single green spectral line of rather low intensity. The effect of irradiation was marked increase in rate of plasmolysis which lasted during the entire experiment. Growth was also markedly retarded at the beginning but a partial recovery occurred. These results show that the wave-length range must have some profound effect on protoplasm apart from the intensity of the line, as λ 4960–4916 Å is one of the least intense lines of the visible spectrum.

λ 4359–4348 Å

This range includes a line of intermediate intensity at the violet end of the visible spectrum. Rate of plasmolysis was slightly increased during the first 6 hr. of irradiation, this increase continuing throughout the 24 hr. The division rate was stimulated continuously over this period.

λ 3821–3752 Å

Probably includes several lines of low intensity in this region. The results for the division rate were most erratic, initial stimulation and retardation both

being recorded. In all cases the rate of plasmolysis was found to be greatly increased for both the 6 and 24-hr. exposures.

λ 3663–3658 Å

Slight stimulation of growth with an increase in plasmolysis rate.

λ 3352–3342 Å

This includes a line of intermediate intensity in the near ultra-violet, which caused a decided increase in the rate of plasmolysis coupled with a decided stimulation of growth for the first 6 hr., which, however, fell off slightly before the completion of the 24-hr. period.

λ 3132 Å

A line of intermediate intensity in the near ultra-violet. Stimulated growth to some extent and increased the rate of plasmolysis throughout the entire experiment.

λ 3028–3022 Å

A decided stimulation of plasmolysis rate coupled with a slight but decided decrease in division rate.

λ 2967 Å, λ 2804 Å, λ 2700 Å, λ 2535 Å and λ 2054 Å

All showed a very decided and consistent increase in the rate of plasmolysis coupled with a continued decrease in growth rate.

Comparison of the Effect of Monochromatic Light on the Rate of Plasmolysis in (i) Sodium Chloride Solutions and (ii) Sucrose Solutions

In general the rate of plasmolysis in sodium chloride solutions was more rapid than in sucrose solutions, even though the latter had a greater molecular concentration. The sodium chloride solutions used were 0.3 and 0.7 *M* and those of sugar, 0.7 and 1.0 *M*. The extreme increase in plasmolysis in the case of sodium chloride solutions was 68% for 2054 Å at 24 hr., whereas the extreme increase in plasmolysis for sucrose solutions was 42% in the case of 5819 Å and 4359 Å. The average increase in plasmolysis for 0.7 *M* sodium chloride solutions was approximately 30% at the end of 6 hr. and for 0.3 *M* sodium chloride was 35%; whereas in the case of sucrose solutions the average plasmolysis for molar solutions was approximately 20% and for 0.7 *M* was less than 30%. The results for plasmolysis in the sucrose solutions after 24 hr. irradiation, especially in the region of visible light, were apparently erratic, possibly because of the presence of a determining factor different from that which seems to limit the rate of plasmolysis in the sodium chloride solutions, either after 6 or 24 hr. irradiation, and the sucrose solutions after 6 hr. irradiation. The nature of this factor is suggested in the discussion.

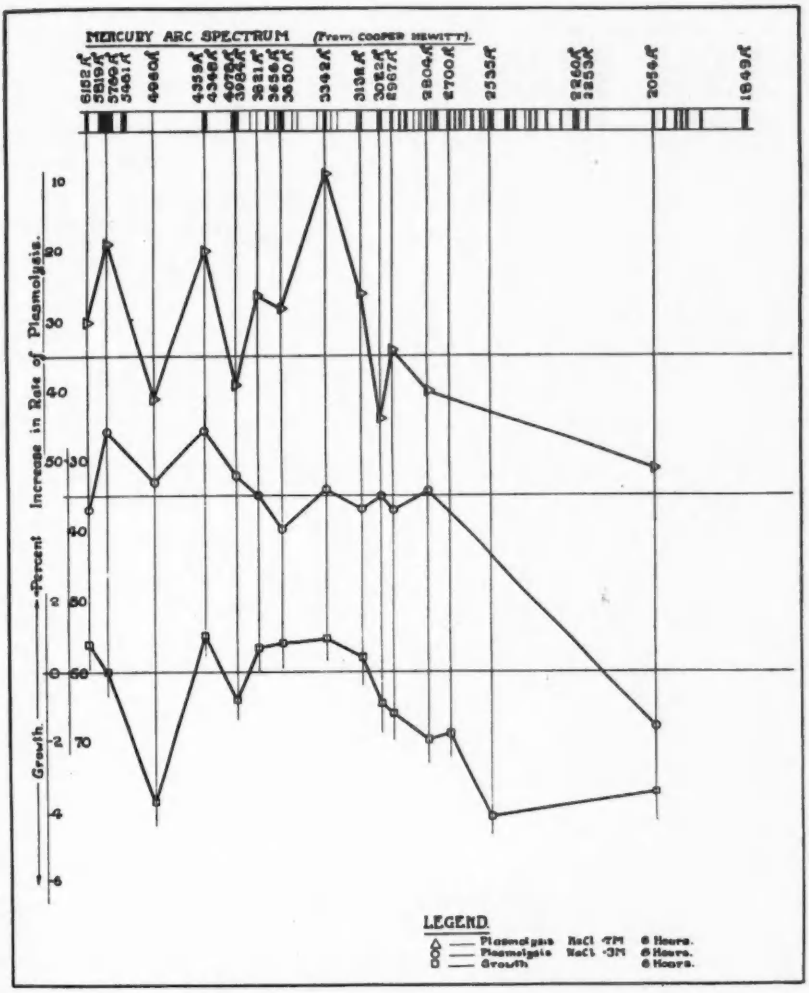


FIG. 1. *Comparison of the effect on growth and on plasmolysis in sodium chloride solutions.*

Comparison of the Rate of Plasmolysis after Irradiation with the Rate of Growth During Irradiation

In general there is a marked similarity between the curves showing the increase in rate of plasmolysis and those showing the effect of irradiation on the growth rate of paramecium (Fig. 1-4). After irradiation for 6 hr. the growth was stimulated by 6152Å, 4359Å, 3821Å, 3656Å and 3342Å, while

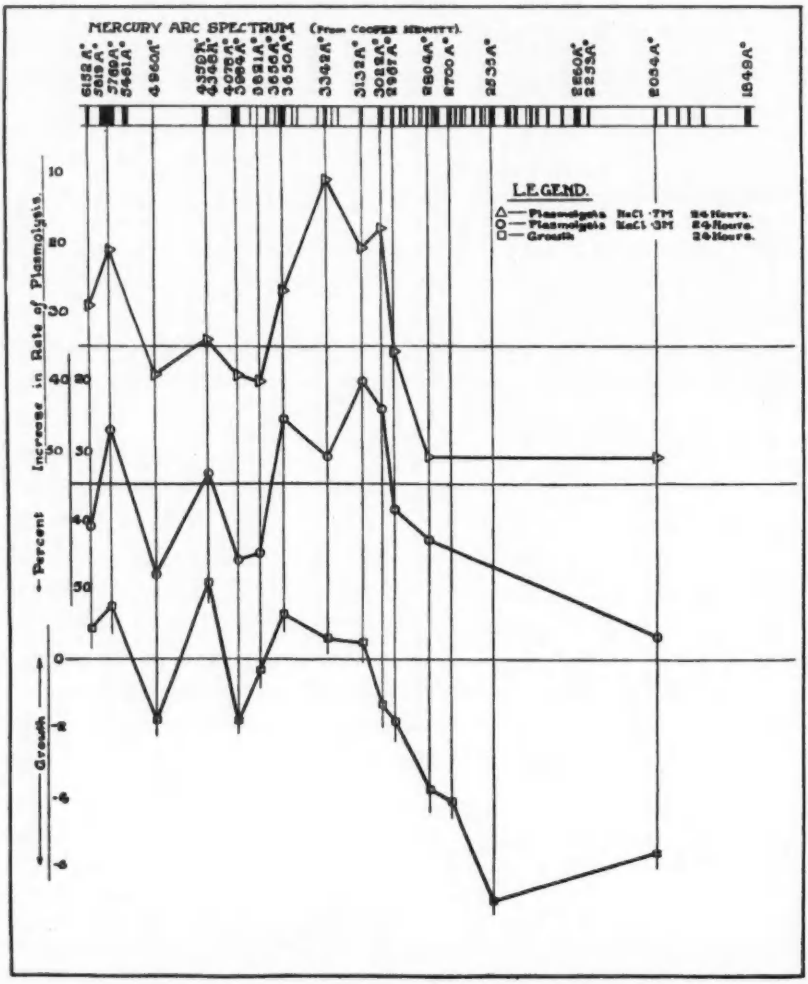


FIG. 2. *Comparison of the effect on growth and on plasmolysis in sodium chloride solutions.*

plasmolysis in 0.7 *M* sodium chloride showed less than 30% increase after irradiation with the same wave-lengths, (with the exception of 6152 Å), and less than 25% increase in rate of plasmolysis in molar sucrose after irradiation by these wave-lengths. Wave-lengths 4960Å, 4078Å, 3132Å, 3022Å, 2967Å, 2804Å, 2700Å and 2054Å all resulted in decrease in the rate of growth for irradiated paramecia. The same lines gave an increase in the rate of plas-

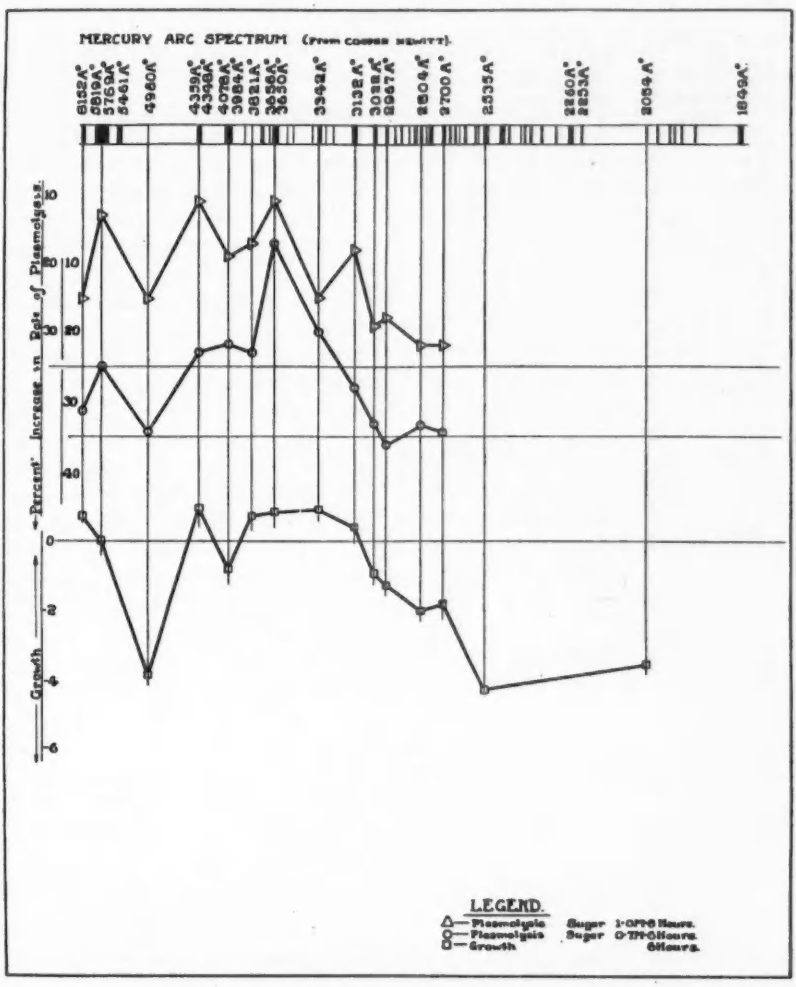


FIG. 3. *Comparison of the effect on growth and on plasmolysis in sucrose solutions.*

molysis which was greater than 35% for 0.7 *M* sodium chloride in all cases, and greater than 23% in molar sucrose, with one exception, 3132 Å.

It would appear that the conditions accompanying moderate increase in rate of plasmolysis also accompany an increase in the rate of cell division in paramecium, and that the conditions accompanying excessive increase in rate of plasmolysis result in decrease in growth. A few deviations from this rule

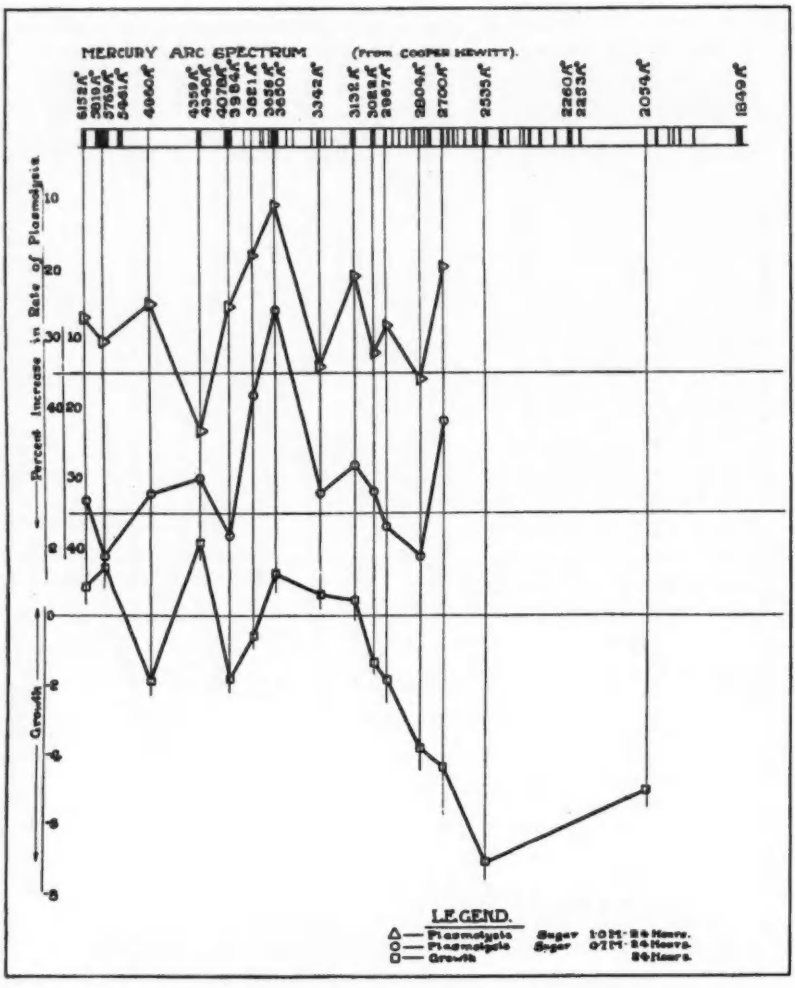


FIG. 4. *Comparison of the effect on growth and on plasmolysis in sucrose solutions.*

appear in the case of paramecium irradiated for 24 hr. when the plasmolysis was determined by placing in 0.7 *M* sucrose (Fig. 4). It would seem that the increase in the permeability towards sucrose is affected differently from that towards the solutes of the cell sap and that this may account for the seemingly erratic results in this particular instance.

Discussion

An increase in the rate of plasmolysis may result from one or several of a number of possible factors, or any one of the series may act as the limiting factor: (i) a decrease in the osmotic pressure of the cell sap caused by increased permeability of the membrane which (a) allows the solutes of the cell to escape by diffusion and (b) decreases semipermeability and consequently endosmotic forces; (ii) an increase in the osmotic pressure of the supernatant solution brought about by a decrease in permeability with respect to the solutes, *i.e.*, an increased semipermeability. It is evident that increased permeability may result in either decrease or increase in the rate of plasmolysis according to whether it has reference to the solutes of the supernatant solution or to those of the cell sap. Since permeability is relative and since a general increase in semipermeability results in increased exosmotic as well as endosmotic forces, it follows that increased rate of plasmolysis involves a relative increase in permeability toward the solutes of the cell sap and a relative decrease toward those of the supernatant liquid. This investigation has not determined the exact nature of these relative changes and consequently the effect has been described in terms of the actual observation; namely, the effect on the rate of plasmolysis. The seemingly erratic results obtained in the visible spectrum with 0.7 *M* sucrose after 24 hr. irradiation may be explained theoretically on a basis of the conditions described above.

References

1. Blackman, V. H. and Paine, S. G. Ann. Botany, 32: 69-85. 1918.
2. Harvey, E. N. Papers from the Tortugas Lab., Dept. Marine Biol., Carnegie Inst. Wash. 6: 133-146. 1914.
3. Hertel, E. Z. allgem. Physiol. 5: 1-43, 95-122. 1905.
4. Hoagland, D. R. and Davis, A. R. J. Gen. Physiol. 4: 47-62. 1923.
5. Hutchinson, A. H. and Ashton, M. R. Can. J. Research, 1: 292-304. 1929.
6. Lepeschkin, W. W. Am. J. Botany, 17: 953-971. 1930.
7. Osterhout, W. J. V. Injury, recovery and death in relation to conductivity and permeability. J. P. Lippincott. 1922.
8. Packard, C. J. Gen. Physiol. 7:363-372. 1925.
9. Sayre, J. D. Plant Physiol. 4: 323-328. 1929.

REVIEWS AND NOTES

CHROMOSOME HOMOLOGIES IN WHEAT, RYE AND AEGILOPS[1]

By W. P. Thompson[2]

Contents

Introduction

A great deal of cytogenetic work has been done recently on numerous interspecific hybrids involving the three related genera *Triticum*, *Secale* and *Aegilops*. The hybrids investigated have been both intra- and intergeneric, and all three generic combinations have been studied. Furthermore, the hybrids have represented nearly all possible combinations of the groups of species into which the genera in question can be divided on the basis of chromosome numbers. The homologies of the chromosomes in the numerous species, as judged by their mating in the hybrids, have been discussed by several authors. The results have appeared in numerous papers widely scattered in the literature of many countries. It is proposed in the present paper to bring together the essential data in so far as they bear on the question of chromosome homologies. No attempt will be made to deal with the numerous other problems involved.

Chromosome Numbers in the Pure Species

The chromosome numbers of the different species of the three genera as determined by numerous authors are given in Table I. There is no question about the correctness of the numbers as given for *Triticum* and for most of the species of *Aegilops*. Differences of opinion regarding certain species of *Aegilops* are apparently due to errors in taxonomic identification. Recently Bleier (3) has given the number in *A. triaristata* as 21. According to Watkins (27) the wild grass called *Triticum villosum* should be classified with *Secale**. *S. cereale* is known occasionally to have eight chromosomes instead of seven.

[1] *Manuscript received March 23, 1931.*
Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Canada.

[2] *Professor of Biology, University of Saskatchewan.*

**Dr. H. T. Gussow informed the author in a recent letter that the correct name is Agropyron villosum (Link, Hort. Berol. 1.31).*

TABLE I

HAPLOID CHROMOSOME NUMBERS OF SPECIES OF WHEAT, *Aegilops* AND RYE

Genus	Number		
	7	14	21
	Einkorns	Emmers	*Vulgares*
Triticum	aegilopoides monococcum villosum	dicoccum dicoccoides durum persicum polonicum turgidum	compactum spelta vulgare
Aegilops	aucheri bicornis caudata comosa longissima speltoides squarrosa uniaristata	triuncialis cylindrica ovata triaristata triuncialis variabilis ventricosa	crassa turcomanica
Secale	cereale montanum		

Chromosome Mating in Hybrids

A. INTRAGENERIC

1. In Wheat

a. Between species with the same chromosome number. The observations with respect to the mating of the chromosomes in hybrids between species of wheat with the same chromosome number are summarized in the first part of Table II. Usually there is complete pairing and all pairs mate normally. (The term "closed" is used in the tables to describe the normal, side-by-side appearance at metaphase, as in pure species; the term "open" refers to the loose, end-to-end association common in wide crosses at metaphase. These terms are preferred to "telo- and para-syndetic" of several authors because the conditions in prophases have rarely been recorded.) These observations indicate that every chromosome of each species of the emmer and of the *vulgare* series has a homologue in every other species of the same cytological group. In a percentage of the mother cells, however, one or rarely two pairs fail to mate (1, 26). The percentage is considerably higher (up to 30 or 40) in hybrids between 21-chromosome species than in those between 14-chromosome species, except in special cases. It is possible that in the *vulgare* series the weak affinity of one pair is associated with specific differences. Even in certain hybrids between varieties of the same species one or two pairs frequently fail to mate (5, 25). According to Aase (1), in *dicoccoides* × *durum* two of the bivalents are usually of the open type.

b. Between species with different numbers. In nearly all the 14×7 hybrids seven pairs are capable of mating, but usually some of the seven and sometimes all of them fail to do so. The amount of mating varies in a single plant, from

TABLE II

CHROMOSOME PAIRING IN WHEAT HYBRIDS

Parent		Parent		Bivalents in F_1			References
No.	Species	No.	Species	Range	Usual	Kind	
21	all	21	all	19–21	21	closed	26
14	all	14	all	12–14	14	closed	1, 26
14	turgidum	7	monococcum	7		closed	20
14	all	7	monococcum	3–7	5, 6	mixed	1, 12, 24
14	several	7	monococcum	0–7	4, 5		16
14	dicoccum	7	aegilopoides	6–7		mixed	15
14	dicoccoides	7	aegilopoides	0–5			3
21	all	14	all	14		closed	numerous
21	vulgare	14	durum	13–14	14	closed	15
21	vulgare	14	durum	12–14	14	closed	1
21	spelta	7	monococcum	0–5	3, 4	open	16, 17
21	spelta	7	aegilopoides	6–10	7		14
21	vulgare	7	monococcum	4–7	5	open	16
21	vulgare	7	monococcum	0–5			3
21	compactum	7	monococcum	0–7	5		16
21	compactum		haploid	0–3	0		4

cross to cross, and possibly with external conditions. But the frequent occurrence of seven bivalents in all recorded cases but one, is taken to indicate that all seven chromosomes of the einkorn series have homologues among the 14 of the emmer series. Accordingly, the set of seven in einkorns may be designated *A*, and the two sets in emmers *A* and *B*. The *A* set of einkorns is evidently somewhat differentiated from that of emmers as shown by the facts (a) that some pairs may not conjoin, and (b) the mating is frequently of the "open" type. The observations of Kihara and Nishiyama (15) indicate that the set in *aegilopoides* is more like the *A* of emmers (at least of *dicoccum*) than is that in *monococcum;* but the results of Bleier (3) lead to the opposite conclusion (at least for *dicoccoides*).

In regard to hybrids of the type 21×14 all the numerous investigators report 14 closed bivalents, except in rare cases of non-conjunction. Evidently all 14 emmer chromosomes have very similar homologues in all species of the *vulgare* series. Therefore the *vulgare* sets must be *A* and *B* and a third set which may be called *C* (Table V).

According to these conclusions, crosses of the type 21×7 are $(A+B+C)\times A$. It is therefore to be expected that seven pairs (the *A*'s) would mate. This has been reported to occur in several crosses, but in others not more than five pairs mate, usually only three or four, and frequently none. All these observations may be taken to indicate that there are homologous sets (*A*'s) in the two series although they have become so differentiated that mating is now difficult and frequently fails. The weakened affinity is further shown by the fact that the mating is of the open type. The relationship between *aegilopoides* and *spelta* appears to be closer than that between *monococcum* and any of the *vulgare* series.

When the results of all three types of crosses (14×7, 21×14, 21×7) are considered together, it is seen that the *A* sets of emmers and *vulgares* are much more closely related to each other than either is to the *A* of einkorns. It is to be noted, also, that two chromosomes, one from einkorns and one from *vulgares*, both of which can readily mate with a third (from emmers), cannot mate with each other, or only with great difficulty. This is the situation with respect to three of the *A* set in einkorns and *vulgares*.

Kihara and Nishiyama (15) have found evidence that the two sets of emmers (*A* and *B*) are not completely differentiated. This evidence consists of the occurrence of as many as three trivalents in hybrids between emmers and einkorns (frequencies not stated). Each of the trivalents is presumably composed of an einkorn and two emmer chromosomes, one from *A* and one from *B*. Although we do not know the exact course of evolution, we may suppose that all sets now existing in wheat are modifications of a single set belonging to one original ancestor. *A* and *B* were at one stage in different derivatives of that ancestor, differentiated along separate lines and were subsequently brought together in emmers by hybridizing. Their differentiation had not proceeded far enough to prevent occasional mating on the part of three of their pairs.

From similar observations on hybrids involving *vulgare* types, the same authors conclude that three chromosomes of the *C* set can still mate occasionally with three from the *B* set. This conclusion is supported by the occasional occurrence of one to three bivalents in the haploid wheat studied by Gaines and Aase (4). It is significant in this connection also that the same number of bivalents occurs rarely in hybrids between *vulgare* and rye (12, 23). These bivalents may therefore be the result of mating between wheat chromosomes. and not involve rye at all. If so the occurrence of one to three bivalents in other wide crosses with *vulgare* may result from autosyndesis, and may not indicate homology between *vulgare* chromosomes and those of the species with which it is crossed.

TABLE III

CHROMOSOME PAIRING IN *Aegilops* HYBRIDS

Parent		Parent		Bivalents in F_1			References
No.	Species	No.	Species	Range	Usual	Kind	
14	cylindrica	14	triuncialis	3–12		½-closed	18
14	cylindrica	14	ventricosa		5–7	closed	18
14	cylindrica	14	ovata	3–8		mixed	1
14	cylindrica	14	ovata	7–13		mixed	18
14	ovata	14	triuncialis	5–11	7–8		13
14	ovata	14	triuncialis	0–7			16
14	ovata	14	ventricosa	3–10	7		13
14	ovata	14	ventricosa	3–7		open	18
14	ovata	7	caudata	7–10			2

2. *In Aegilops*

All the crosses within this genus which have been investigated cytologically are shown in Table III. It is clear that the chromosomes of the tetraploid species of *Aegilops* are much more diversified than those of tetraploid wheats. *Cylindrica* and *ventricosa* appear to have one set in common, but the second sets are quite unrelated. *Cylindrica* and *triuncialis* also have one set in common as judged by the closed pairs, and the other sets sufficiently related to permit loose mating at times. Owing to differences in the reported observations, it is difficult to interpret the situation in hybrids involving *ovata*, but it appears that this species has at least one set with weakened affinity for a set in each of the other species. The large number of bivalents frequently observed as well as the common occurrence of trivalents indicate that the second sets also may not be completely differentiated.

TABLE IV

CHROMOSOME PAIRING IN *Aegilops* × *Triticum*

Aegilops		*Triticum*		Bivalents in F_1			References
No.	Species	No.	Species	Range	Usual	Kind	
21	crassa	21	all species	0–7	4–7		16
21	triaristata	21	vulgare	0–7		open	3
21	crassa	14	four species	0–4, 5	0		16
21	crassa	14	dicoccoides	0–5	4		16
14	cylindrica	21	all species	5–7	7	closed	1, 2, 4, 7, 18, 22
14	ovata	21	all species	0–3	0	open	2, 4, 18
14	ovata	21	compactum	0–3	0		1, 18
14	ovata	21	spelta	0–3	1, 2	open	1, 18
14	triuncialis	21	vulgare	0–3		open	1
14	triuncialis	21	vulgare	0–5	1, 3	open	13
14	triuncialis	21	vulgare	1–5			18
14	cylindrica	14	five species (11 reports)	0, 1–4	0	open	1, 2, 4, 8, 16, 18
14	ovata	14	five species (9 reports)	0–2, 3	0	open	1, 2, 4, 8, 13, 18, 21
14	ovata	14	durum	0			4
14	ovata	14	durum	0–4	0, 1	open	1
14	ovata	14	durum	0–7	0–5	open	13
14	ovata	14	durum	1–2	1	open	18
14	triuncialis	14	dicoccum	1–7	4, 5	open	13
14	triuncialis	14	dicoccoides	0–7	2–4		13
14	triuncialis	14	dicoccoides	1–3		open	18
14	triuncialis	14	durum	0–8	4–6	open	13
14	triuncialis	14	durum	1–6			18
14	triuncialis	14	polonicum	3–8	5, 6		13
14	triuncialis	14	turgidum	1–3		open	18
14	ventricosa	14	four species	0–2	rare	open	18
14	ovata	7	monococcum	0–5, 6	1–3		1, 3, 18
14	ovata	7	villosum	0			2
14	ventricosa	7	monococcum	1–4		mixed	18
14	ventricosa	7	villosum	?–4	4		2
7	speltoides	14	turgidum	6–10	7	open	6

3. In Rye

Both *Secale cereale* and *S. montanum* have seven chromosomes, and in the hybrid between them Longley and Sando (16) found seven bivalents as a rule, but occasionally observed non-conjunction of one pair. Though the type of bivalents was not stated it may be concluded that there is complete homology.

B. Intergeneric

1. Wheat × Aegilops

The observations on the numerous hybrids of this kind which have been investigated are collected in Table IV. The conclusions to which these observations lead, if we assume that the occurrence of approximately seven bivalents establishes the presence of homologous sets, will first be discussed, and later the difficulties will be pointed out.

A. cylindrica shows the clearest relationship to wheat. The seven closed bivalents in hybrids with all members of the *vulgare* wheat series (third section of Table IV) show that it has one set in common with *vulgares*. The usual absence of bivalents in hybrids with emmers and the very small number when present show that this set cannot be *A* or *B*. Therefore it must be *C* (see Table V). And the other set in *A. cylindrica* which is evidently different from all wheat sets may be designated *D*. It is well known that Percival concluded from the botanical relationships before any cytological work had been done on these hybrids that the characters which distinguish *vulgares* from emmers had come from an *Aegilops* through hybridizing. It has been suggested that the seven bivalents in *vulgares* × *cylindrica* may be the result of autosyndesis of the *cylindrica* chromosomes, but if so the same seven should appear in emmers × *cylindrica;* that is not the case.

TABLE V

Inferences from chromosome pairing regarding homologies of chromosome sets

Einkorns	A				
Emmers	A + B				
Vulgares	A + B + C				
A. cylindrica		C + D			
A. ovata			D + E		
A. triuncialis	A (or B)	+ D			
A. ventricosa			D +	F?	
A. speltoides	A (or B)				
A. crassa		C		++	
A. caudata			D (or E)		
Secale			D		

A. ovata can have none of the wheat sets *A*, *B*, or *C*, since there is almost always no mating in its hybrids with either emmers or *vulgares*. But it has a set in common with *A. cylindrica* (Table III). This must be *D*. The other *ovata* set must be different from all these and may therefore be designated *E* (Table V). Percival's (18) observations on *ovata* × *cylindrica* would indicate that *E* is sufficiently like *C* to permit occasional loose mating, but Aase's (1) results are not in agreement with this. The latter author suggests that *ovata*

may be an autotetraploid; if so more bivalents would be expected in its hybrids both with *vulgares* and with emmers, and Kagawa's studies (10) on chromosome morphology in *A. ovata* are decidedly opposed to an autotetraploid interpretation.

A. triuncialis appears to have a set in common with emmers (fourth section of Table IV); if so it must be *A* or *B*. Its second set must then be *D* since it has a set in common with both *A. cylindrica* and *A. ovata*, and *D* is the only one which satisfies this condition.

A. ventricosa obviously lacks the *A* and *B* of emmers but has one set in common with *ovata* and one with *cylindrica*. If the same *ventricosa* set mates with one from *ovata* and with one from *cylindrica* it must be *D*; in this case the second *ventricosa* set must be new (*F*). If different *ventricosa* sets mate with chromosomes from *ovata* and from *cylindrica* they must be *C* and *E*.

The usual occurrence of four to seven bivalents in crosses between the 21-chromosome *A. crassa* and *vulgare* wheats may indicate an homology of one set. But this number of bivalents may also result from the autosyndesis of wheat chromosomes on the one hand and of *Aegilops* ones on the other, since there are 21 from each parent. If there is a real homology, the set in question cannot be *A* or *B* as may be seen from the lack of mating in emmer hybrids.

From the conditions in *A. ovata* × *A. caudata* it may be concluded that *caudata* has one of the *ovata* sets (*D* or *E*), and from *T. turgidum* × *A. speltoides* that the latter has *A* or *B*.

TABLE VI

CHROMOSOME PAIRING IN HYBRIDS BETWEEN WHEAT AND RYE AND BETWEEN *Aegilops* AND RYE

Wheat		Rye		Bivalents in F_1			Reference
No.	Species	No.	Species	Range	Usual	Kind	
21	vulgare	7	cereale	0–3	0	open	12, 23
21	vulgare	7	cereale	0–3	0, 1	open	1
21	vulgare	7	cereale	0–4	0	open	3
21	vulgare	7	cereale	0			16
21	vulgare	7	montanum	0–1	0		16
21	spelta	7	cereale	0–4	0		1
21	spelta	7	montanum	0–3	0		16
14	dicoccoides	7	montanum	0			16
14	durum	7	cereale	0–4	1	open	1
14	durum	7	cereale	0–2			19
14	persicum	7	cereale	0–2			19
14	aegilops triuncialis	7	cereale	5–7	5	open	11

2 and 3. Wheat × Rye and Aegilops × Rye

Several investigators have obtained consistent cytological results in hybrids between *vulgare* wheats and rye (Table VI). The occasional bivalents are apparently not more numerous than in haploid wheat. They may therefore be regarded as the results of autosyndesis, and not as an indication of homology

as between wheat and rye chromosomes. The same statements apply to the emmer-rye hybrids. Rye therefore does not possess *A*, *B* or *C*. The regular mating of five pairs and frequent mating of seven in *A. triuncialis* × rye would indicate that the rye set is *D*. It is conceivable that these bivalents result from autosyndesis of *triuncialis* chromosomes but if so, as many should be observed in *A. triuncialis* × *T. vulgare*.

Difficulties and Objections

The method of interpreting chromosome homologies from the number and kind of bivalents in F_1 meets with several difficulties in these genera. In many cases the amount of mating in individual plants or stamens is so variable that its real meaning is doubtful. In several instances the reports of different authors do not agree. It is possible that the differences are due in part to external influences. Kihara (13) found that material of the same cross fixed at different times showed quite different numbers of bivalents, as did different florets fixed at the same time. Although he did not determine the external influence which was responsible for such differences, it is known from recent work on several other plants that differences in temperature may have marked effects on the amount of mating. If such external influences affect mating which is at best highly variable, caution must be exercised in drawing inferences respecting homologies.

Even when there is substantial agreement in the reports of different investigators it is difficult or impossible in some cases to decide how the results should be interpreted. This is particularly the case when only a few chromosomes, or when more than seven pair consistently (*e.g.*, *A. crassa* × *T. dicoccoides*, *A. ovata* × *A. triuncialis*). In such cases and in others, chromosomes from the same parent may have been mated. There is good evidence (15) that two or three chromosomes from the *A* set may mate with ones from *B* in emmers when conditions are appropriate, and that two or three from *B* may mate with *C* in *vulgares*. If this occurs in respect to the chromosomes from both parents, a sufficient amount of mating is produced to give the false impression that two homologous sets are present. On the other hand there is little evidence that any of the species in question are true autopolyploids as Percival suggested for *T. dicoccoides*, and Aase for *A. ovata*. If that were true all hybrids of these species would show at least seven bivalents; this is not the case.

The changes, which in the course of evolution have weakened the capacity to mate on the part of chromosomes which were originally fully homologous, may have been changes in genes or in chromosome structure, *i.e.* translocations, deletions, etc. It is not conceivable that these changes should have affected all chromosomes of a set simultaneously. We should therefore expect that some members of diverging sets would have retained the capacity to mate, while others would not. Accordingly there is no reason to expect that all the chromosomes of all the species could be divided consistently into sets of seven. Where this can be done the species in question are closely related. The

exchange of segments of non-homologous chromosomes, such as is known to have occurred in other plants including *Datura* and maize, might cause the loose type of mating so common in these cereal hybrids.

Another difficulty is found in the inconsistent conclusions which may be drawn from the results of crossing the same group of species in different combinations. On the basis of chromosome mating in its hybrids with *vulgares* and emmers, *A. ovata* has neither *A* nor *B*, but on the basis of crosses with *monococcum* it has *A* (or most of it). There is a similar situation with respect to *A. ventricosa*. The chromosomes of *A. crassa* appear to have much greater affinity for those of *T. dicoccoides* than for those of other emmers, but all chromosomes of *T. dicoccoides* mate with those of other emmers. According to one observer *A. cylindrica* can form as many as 13 bivalents in combination with *A. ovata*, but one of these species can form seven and the other none at all in combination with *T. vulgare*.

When genetic points are taken into consideration, fresh difficulties arise. On the basis of mating with chromosomes of *T. turgidum*, the single set of *A. speltoides* must be either *A* or *B*. But it carries several of the distinctly *vulgare* characters which are supposed to be in *C*. Genetically it is *C*, cytologically *A* or *B*. To judge by mating in hybrids with *A. triuncialis*, rye chromosomes are *D*, but the characters are not those expected on the basis of the other occurrences of *D*.

It is possible that in many cases the two chromosomes associated at metaphase in a loose end-to-end fashion do not constitute a true bivalent. They may have been attached end-to-end in the earlier spireme stage, and in the absence of true pairing may have remained associated longer than usual.

It is also possible that where the total number is large, although a set from each parent may be the same, they have difficulty in mating owing to the presence of a large number which have no partners; or chromosomes which could mate in some cytoplasmic conditions may be unable to do so in others.

Chromosome Morphology

Chromosome homologies may be inferred not only from mating capabilities but also from their morphological resemblances and differences. The only investigator who has reported any success in distinguishing individual wheat chromosomes is Kagawa (9, 10). On the basis of their relative lengths and of the

TABLE VII

NUMBERS OF CHROMOSOME PAIRS OF VARIOUS TYPES IN CERTAIN SPECIES OF *Triticum* (ACCORDING TO KAGAWA)

Species	I	II	IIa	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
monococcum	1	1		1	1	3											
polonicum	1		1		1	6	1	2	1	1							
dicoccum	1	1			1	5		1	1	1	1	1	1				
vulgare	1					13		1	1	1				1	1	1	1

number and position of their constrictions, Kagawa distinguishes 17 types of chromosomes in certains species of wheat, as shown in Table VII. These conclusions are not in agreement with those reached from observations on mating. If morphological similarity and mating capabilities both indicate homology, morphologically similar chromosomes should pair. Nevertheless several morphological types in *dicoccum* are not present in *polonicum* although every chromosome in *dicoccum* mates closely with one in *polonicum*. Similarly several types in *dicoccum* and *polonicum* are not present in *vulgare* although every chromosome of each of the first two finds a mate among those of the last named species. Again three types in *monococcum* are unlike any in *vulgare*, but Longley and Sando found that every chromosome in *monococcum* can mate at least occasionally with a chromosome from *vulgare*. Evidently if Kagawa's conclusions are well founded, homology as judged by chromosome morphology, is different from homology as judged by mating.

Conclusions

In view of all the difficulties, disagreements, inconsistencies and possible sources of error much more work must be done before a satisfactory scheme of chromosome homologies, embracing all the species and reflecting botanical and genetical relationships, can be drawn up. Except in special instances involving closely related types, therefore, phylogenetic speculation would be premature. The work already done, however, does establish securely certain important points (upper part of Table V) and gives promise that at least much of the tangle will eventually be unravelled. The study of certain critical crosses and of crosses involving species not yet studied should solve many difficulties. For the investigation of chromosomal and genetic changes in a large series of related types which have undergone just sufficient evolutionary divergence to present all degrees of specific and varietal difference, the three genera appear to present excellent material.

References

1. Aase, H. C. Research Studies, State College of Washington, 2: 1-60. 1930.
2. Bleier, H. Verhandl. V. internat. Kongr. Vererb. 1: 447-452. 1927.
3. Bleier, H. Der Zuchter, 2: 12-22. 1930.
4. Gaines, E. F. and Aase, H. C. Am. J. Botany, 13: 373-385. 1926.
5. Goulden, C. F. Univ. Minnesota Tech. Bull. 33. 1926.
6. Jenkins, J. A. Am. J. Botany, 16: 238-245. 1929.
7. Kagawa, F. Japan. J. Botany, 4: 1-23. 1928.
8. Kagawa, F. Japan. J. Botany, 4: 345-360. 1929.
9. Kagawa, F. Japan. J. Botany, 4: 363-382. 1929.
10. Kagawa, F. J. Coll. Agr. Imp. Univ. Tokyo, 10: 174-227. 1929.
11. Karpechenko, G. D. and Sorokina, O. N. Bull. Applied Botany, 563-584. 1929.
12. Kihara, H. Mem. Coll. Sci. Kyoto Imp. Univ. 1: 1-200. 1924.
13. Kihara, H. Cytologia, 1: 1-15. 1929.
14. Kihara, H. and Nishiyama, I. Botan. Mag. 12: 221-231. 1928.
15. Kihara, H. and Nishiyama, I. Cytologia, 1: 263-284. 1930.
16. Longley, A. E. and Sando, W. J. J. Agr. Research, 40: 683-719. 1930.

17. MELBURN, M. C. and THOMPSON, W. P. Am. J. Botany, 14: 327-333. 1927.
18. PERCIVAL, J. J. Genetics, 22: 201-278. 1930.
19. PLOTNIKOWA, T. W. Z. wiss. Biol. 12: 167-183. 1930.
20. SAX, K. Genetics, 7: 513-552. 1922.
21. SAX, K. Verhandl. V. internat. Kongr. Vererb. 2: 1267-1284. 1927.
22. SAX, K. and SAX, H. J. Genetics, 9: 454-464. 1924.
23. THOMPSON, W. P. Genetics, 11: 317-332. 1926.
24. THOMPSON, W. P. J. Genetics, 17: 43-48. 1926.
25. THOMPSON, W. P. Trans. Roy. Soc. Canada, 22: 335-348. 1928.
26. THOMPSON, W. P. and ROBERTSON, H. T. Cytologia, 1: 252-262. 1930.
27. WATKINS, A. E. J. Genetics, 23: 173-263. 1930.